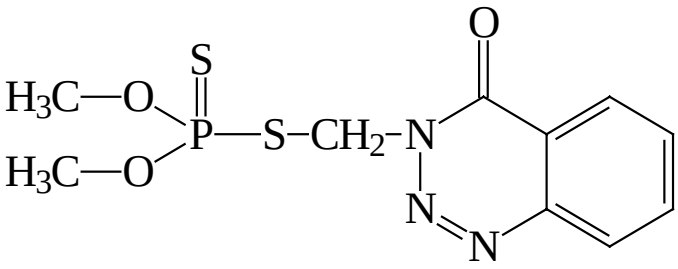
EVALUATION OF AZINPHOS-METHYL AS A TOXIC AIR CONTAMINANT



Part C

Human Health Assessment

California Department of Pesticide Regulation
Medical Toxicology Branch

California Environmental Protection Agency
Sacramento, California

July 2000

California Environmental Protection Agency
Department of Pesticide Regulation

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I. SUMMARY

This document addresses the potential human health effects from exposure to azinphos-methyl in offsite and ambient air due to its agricultural use. Azinphos-methyl is a broad spectrum organophosphate insecticide, acaricide, and molluscacide whose primary use in California is on tree crops such as almonds, pears, walnut, apples, peaches, and pistachios. Azinphos-methyl and its oxygen analog produce their toxic reaction primarily through their inhibition of acetylcholinesterase (AChE) which is responsible for terminating transmission of impulses across certain nerve synapses.

The primary effects observed in laboratory animals from acute exposure to azinphos-methyl are cholinergic signs including piloerection, ocular and nasal discharge, salivation, breathing difficulties, staggering gait, tremors, twitching, and/or convulsions. The lowest established no-observed-effect level (NOEL) for overt toxicity in an acceptable acute toxicity study in animals was 1.0 mg/kg based on reduced performance in the functional observational battery (sitting/lying in open field, reduced approach response, and uncoordinated righting response) and brain cholinesterase (ChE) inhibition in female rats administered a single dose of azinphos-methyl by oral gavage. A NOEL for red blood cell (RBC) ChE inhibition was not established in this study, but was estimated to be 0.1 mg/kg. A NOEL for plasma and RBC ChE inhibition was established in a human study at 0.75 mg/kg. With subchronic exposure to azinphos-methyl, cholinergic signs, ChE inhibition, reduced body weights, impaired spermatogenesis, and decreased pup viability and lactation indices were seen in laboratory animals. The lowest NOEL in an acceptable subchronic study was estimated to be 0.09 mg/kg/day based on plasma, RBC and brain ChE inhibition in rats. In the chronic studies in laboratory animals, cholinergic signs, reduced body weights, ChE inhibition and cystic endometrial hyperplasia were seen. The lowest chronic NOEL in an acceptable study was 0.15 mg/kg/day based on diarrhea and RBC ChE inhibition in dogs.

There was limited evidence that azinphos-methyl may be genotoxic and oncogenic. There was an increase in tumors of the pancreas, thyroid, and adrenal glands of males in a rat oncogenicity study. An increase in liver tumors was also seen in males in a mouse oncogenicity study. However, both of these studies had an inadequate number of concurrent control animals that made interpretation of these findings difficult. Furthermore, there was no increase in these tumors in well-conducted oncogenicity studies in both species. Azinphos-methyl was positive in selected in vitro genotoxicity assays, but in none of the in vivo assays. DPR toxicologists concluded that the limited evidence of oncogenicity in animals for azinphos-methyl was insufficient to warrant further evaluation for oncogenic potential.

The absorbed daily dosages (ADDs) for offsite (application site) air were based on air monitoring following an application to a walnut orchard in Glenn County. The ADDs for offsite air were 80 and 170 ng/kg for adults and children, respectively. The ADDs for ambient air were based on air monitoring conducted in five rural locations in Kern County during one month. The ADDs were initially calculated for the Pond site which had the highest average and 95th percentile air concentrations of azinphos-methyl. The ADDs for ambient air at the Pond site ranged from 23.1 ng/kg for adult females to 61.3 ng/kg for children based on the 95th percentile

air concentration. The seasonal average daily dosages (SADDs) for ambient air at the Pond site ranged from 4.7 ng/kg/day for adult females to 11.4 ng/kg/day for children based on the average air concentration during the monitoring period. The annual average daily dosages (AADDs) for ambient air at the Pond site ranged from 1.9 ng/kg/day for adult females to 4.7 ng/kg/day for children, assuming potential exposure of 180 days per year. Due to their higher respiratory rate relative to their body weight, children consistently had the highest exposure.

The risk for non-oncogenic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL in animal studies to the human exposure dosage. Generally, an MOE greater than 100 is desirable when the NOEL is based on animal data. When the NOEL is based on human data, an MOE greater than 10 is generally desirable. The MOEs for acute exposure to azinphos-methyl in offsite and ambient air ranged from 590 to 64,000 depending on the NOEL used and the population subgroup. The MOEs for seasonal exposure to azinphos-methyl in ambient air ranged from 7,900 to 19,000. The MOEs for chronic exposure azinphos-methyl in ambient air were between 32,000 and 79,000.

Air concentrations of azinphos-methyl that are below the reference concentrations (RfCs) are considered sufficiently low to protect human health. The acute RfCs for azinphos-methyl range from 1.3 $\mu g/m^3$ (0.10 ppb) to 101 $\mu g/m^3$ (7.8 ppb) depending on which NOEL was used. The seasonal RfC is 1.2 $\mu g/m^3$ (0.09 ppb) based on plasma, RBC and brain ChE inhibition in rats. The chronic RfC is 2.0 $\mu g/m^3$ (0.16 ppb) based on diarrhea and RBC ChE inhibition in dogs.

II. INTRODUCTION

A. Regulatory History

Azinphos-methyl (O,O-dimethyl-S-[(4-oxo-1,2,3-benzotriazin-3(4H)yl)methyl] phosphorodithioate) is a broad spectrum organophosphate insecticide, acaricide, and molluscacide whose primary use in California is on tree crops such as almonds, pears, walnut, apples, peaches, and pistachios. Azinphos-methyl was first registered in 1959 by Mobay Chemical Corporation in the United States (U.S. EPA, 1986). In 1986, the U.S. EPA issued a reregistration standard for azinphos-methyl. The Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency placed azinphos-methyl on the high-priority list for risk assessment based on possible adverse effects identified in chromosomal aberrations and oncogenicity studies submitted under the Birth Defect Prevention Act (SB 950) and due to its low no-observed-effect level (NOEL) for acute toxicity. Azinphos-methyl is a restricted-use pesticide based on its acute toxicity. In 1993, the U.S. EPA issued an acute data call-in for illness reports from poison control centers because of concerns regarding acute risks to human health. Azinphos-methyl is also a high-priority pesticide for risk assessment under the California Toxic Air Contaminant Act (AB 1807). A Risk Characterization Document (RCD) for azinphos-methyl has been completed (dated April 29, 1998) by DPR which addresses the occupational and dietary exposure. The environmental fate and air monitoring data for azinphos-methyl were summarized in Part A (Environmental Fate) of the Evaluation of Azinphos-Methyl as a Toxic Air Contaminant. Based on data presented in Part A, exposure to azinphos-methyl in offsite and ambient air was estimated in Part B (Exposure Assessment) of the Evaluation of Azinphos-methyl as a Toxic Air Contaminant. This document, Part C (Health Assessment) of the Evaluation of Azinphos-methyl as a Toxic Air Contaminant addresses the potential health effects in the general public from exposure to azinphos-methyl in offsite and ambient air due to its agriculture use.

B. Mechanism of Action

Azinphos-methyl and its oxygen analog produce their toxic reaction primarily through their inhibition of cholinesterase (ChE) enzymes. ChEs are a family of enzymes found throughout the body that hydrolyze choline esters. Acetylcholinesterase (AChE; also called specific or true cholinesterase) is found near cholinergic synapses, in some organs (e.g. lung, spleen, gray matter) and in red blood cells (Lefkowitz *et al.*, 1990). Normally, AChE metabolizes acetylcholine to acetate and choline, which results in the termination of stimulation to dendritic nerve endings and motor endplates. Acetylcholine is the neurochemical transmitter at endings of postganglionic parasympathetic nerve fibers, somatic motor nerves to skeletal muscle, preganglionic fibers of both parasympathetic and sympathetic nerves, and certain synapses in the central nervous system (CNS) (Murphy, 1986).

The inhibition of AChE results in the accumulation of endogenous acetylcholine in nerve tissue and effector organs. In acutely toxic episodes, muscarinic, nicotinic and CNS receptors are stimulated with characteristic signs and symptoms occurring throughout the peripheral and central nervous systems (Ellenhorn and Barceloux, 1997; Murphy, 1986). Muscarinic effects can

include increased intestinal motility, bronchial constriction and increased bronchial secretions, bladder contraction, miosis, secretory gland stimulation and bradycardia. Nicotinic effects include muscle weakness, twitching, cramps and general fasciculations. Accumulation of acetylcholine in the CNS can cause headache, restlessness, insomnia, anxiety and other non-specific symptoms. Severe poisoning results in slurred speech, tremors, ataxia, convulsions, depression of respiratory and circulatory centers and, eventually, coma.

Butyrylcholinesterase (BuChE), sometimes referred to as plasma ChE, pseudo-cholinesterase, or serum esterase, is also inhibited by azinphos-methyl. Any reference in this document to "cholinesterase", without specifically indicating that the enzyme is serum or plasma ChE, should be interpreted as AChE. BuChE only occurs to a limited extent in neuronal elements of the central and peripheral nervous systems in adults, but it appears to be important in the developing nervous system of birds and mammals where it is the predominant form of cholinesterase (Brimijoin and Koenigsberger, 1999). As neuroblasts switch from cell proliferation to neural differentiation, there is concomitant switch from BuChE to AChE. Unlike AChE, BuChE occurs primarily in non-neuronal or non-synaptic sites in adults like the liver, lung, and plasma and has no known physiological function (Lefkowitz *et al.*, 1990; Brimijoin, 1992; U.S. EPA, 1993; Pantuck, 1993). An atypical genetic variant of plasma cholinesterase has been associated with an increased susceptibility to various drugs, such as succinylcholine and cocaine (Lockridge, 1990; Pantuck, 1993). However, it is unclear if this increased susceptibility to certain drugs in people with the atypical plasma ChE translates to a possible adverse effect when plasma ChE is inhibited by organophosphates. In an *in vitro* study, it was shown that the atypical and normal plasma ChE were equally sensitive to the organophosphate inhibitors, diisopropylfluorophosphonate (DFP) and tetraethylpyrophosphonate (TEPP), but the atypical plasma ChE was less sensitive than the normal plasma ChE to 14 drugs, especially succinylcholine and decamethonium (Kalow and Davies, 1958). In another study, rats that were depleted of plasma AChE by injecting them intravenously with antibodies specific to this enzyme were not more susceptible to paraoxon toxicity than untreated controls based on their performance in a functional observational battery and AChE activity in the brain and diaphragm (Padilla et al., 1992). On the other hand, administration of exogenous BuChE has been demonstrated to provide significant protection against several organophosphate compounds in rats, mice, guinea pigs and non-human primates (Raveh *et al.*, 1997; Allon *et al.*, 1998).

At 0.1 mM, azinphos-methyl also inhibits the active transport of glucose in isolated mouse intestine (Guthrie *et al.*, 1974). The mechanism by which it inhibits glucose transport is unknown. It is also unknown if this *in vitro* biochemical effect has any relationship to clinical or pathological effects observed *in vivo*.

III. METABOLISM AND PHARMACOKINETICS

A. Introduction

Two studies of the metabolism and pharmacokinetics of azinphos-methyl were conducted in rats by the registrant (Patzschke *et al.*, 1976; Kao, 1988). Several other published metabolism/pharmacokinetic studies were conducted in rats and other species, but were not guideline-type studies.

B. Absorption

Oral: Azinphos-methyl, administered to rats, cattle and chickens by the oral route, was rapidly absorbed (Anderson *et al.*, 1974; Patzschke *et al.*, 1976; Kao, 1988; Everett *et al.*, 1966; Scheele *et al.*, 1977). Oral absorption appears to be nearly complete 2-6 hours post-dosing in these three species at which time the maximal blood concentrations are reached. The oral absorption rate was estimated to be 90-100%.

Dermal: The dermal absorption rate of azinphos-methyl in humans was approximately 16% based on a study with male volunteers (Feldman and Maibach, 1974). Radiolabeled azinphos-methyl was applied unoccluded in a 0.25% acetone solution to the forearms of one group, while another group was given the compound intravenously. Approximately 70% of the dose was excreted in the urine within 5 days after intravenous administration of azinphos-methyl. Only 16% was excreted in the urine when applied topically after correcting for the incomplete urinary excretion when administered intravenously.

Inhalation: No data were available on the respiratory uptake and absorption of azinphos-methyl; therefore, a default assumption of 100% was used.

C. Distribution

Forty-eight to 72 hours after oral administration of azinphos-methyl, less than 5% of the total dose remained in the tissues of rats (Patzschke *et al.*, 1976; Kao, 1988). The highest residue levels were in liver and kidneys of rats, cattle, goats, and chickens (Patzschke *et al.*, 1976; Kao, 1988; Everett *et al.*, 1966; Gronberg *et al.* 1988; Ridlen and Pfankuche, 1988). The residue levels in these highly perfused tissues may be related to the apparent binding of azinphos-methyl to hemoglobin (Patzschke *et al.*, 1976). With the exception of RBCs, there was a 10-fold decrease in tissue levels of rats from 6 to 48 hrs after application. There was no difference in the disposition and metabolism of azinphos-methyl between sexes of rats (Kao, 1988).

D. Biotransformation

The first evidence to suggest that azinphos-methyl required metabolic activation to produce its cholinergic effects was the marked difference in its anticholinesterase activity *in vitro* and *in vivo* (DuBois *et al.*, 1957a; Murphy and DuBois, 1957; March *et al.*, 1957; Dahm *et al.*, 1962). These studies indicated that its activation is rapid and occurs primarily in the microsomal

fraction of liver. The active metabolite was identified as the oxygen analog of azinphos-methyl. The concentration of the oxygen analog required to inhibit 50% of rat brain cholinesterase *in vitro* was several orders of magnitude lower than of the parent compound (Dahm *et al.*, 1962). Subsequently, *in vitro* and *in vivo* experiments with mice and rats have shown that the metabolism of azinphos-methyl is primarily due to mixed function oxidases (MFOs) and glutathione (GSH)-transferases in the liver (Motoyama and Dauterman, 1972; Lin *et al.*, 1980; Kao, 1988). Kao (1988) proposed a metabolic pathway for azinphos-methyl (Figure 1) which involved oxidation by MFOs resulting in the formation of azinphos-methyl oxygen analog, benzazimide, and a possible intermediate metabolite, mercaptomethylbenzazimide. Further methylation and oxidation of mercaptomethylbenzazimide generated methylthiomethyl-benzazimide and its corresponding sulfoxide and sulfone. Metabolism of azinphos-methyl by GSH transferases resulted in the formation of desmethyl isoazinphos-methyl and glutathionyl methylbenzazimide. Further hydrolysis and oxidation led to the formation of cysteinylmethyl-benzazimide and its corresponding sulfoxide and sulfone. Piperonyl butoxide administered 1 hr prior to azinphos-methyl inhibited its oxidative desulfuration and oxidative cleavage (Levine and Murphy, 1976). Detoxification of azinphos-methyl by glutathione conjugation increased with the inhibition of oxidative metabolism; however, no significant detoxification of the oxygen analog occurred by glutathione conjugation. The metabolism in cattle, goats, and chickens appear to be similar to rats (Everett *et al.*, 1966; Gronberg *et al.*, 1988; Ridlen and Pfankuche, 1988). The toxicity of the various metabolites is unknown except for benzazimide and methyl benzazimide whose LD_{50} values are at least an order of magnitude larger than the parent compound (see Acute Toxicity section).

The major metabolites in tissues of goats and chickens were identified. In goats, the major metabolites identified in liver, kidney, muscle, fat and milk were (in decreasing order of prevalence) methylthiomethylbenzazimide sulfone, methylbenzazimide-type protein conjugates and methylthiomethylbenzazimide sulfoxide (Gronberg *et al.*, 1988). In chickens, the major metabolites in liver, kidney, muscle, fat, and eggs were (in decreasing order of prevalence) benzazimide, methylthiomethylbenzazimide and its sulfoxide and/or sulfone, azinphos-methyl, and mercaptomethylbenzazimide protein or glucuronide conjugate (Ridlen and Pfankuche, 1988). The difference in metabolite patterns between these two species may be partly due to the difference in the time between the last dose and their sacrifice. The chickens were sacrificed only 2 hrs after their last dose whereas the goats were sacrificed 17-18 hrs after their last dose. One would expect that within a few hours of dosing some of the parent compound would not have been metabolized and many of the metabolites would not have been conjugated.

Metabolites found in the urine after oral administration in rats were cysteinylmethyl-benzazimide sulfoxide and sulfone, methylsulfonylmethylbenzazimide, methylsulfinylmethyl-benzazimide, glutathionyl methylbenzazimide, desmethyl isoazinphos-methyl, benzazimide, and cysteinylmethylbenzazimide (Ecker, 1976; Kao, 1988). The metabolites identified in feces were desmethyl isoazinphos-methyl, azinphos-methyl oxygen analog, methylsulfonylmethyl-benzazimide, cysteinylmethylbenzazimide sulfoxide, and methylthiomethylbenzazimide. No parent compound or its glucuronic or sulfate conjugates were found in urine or feces.

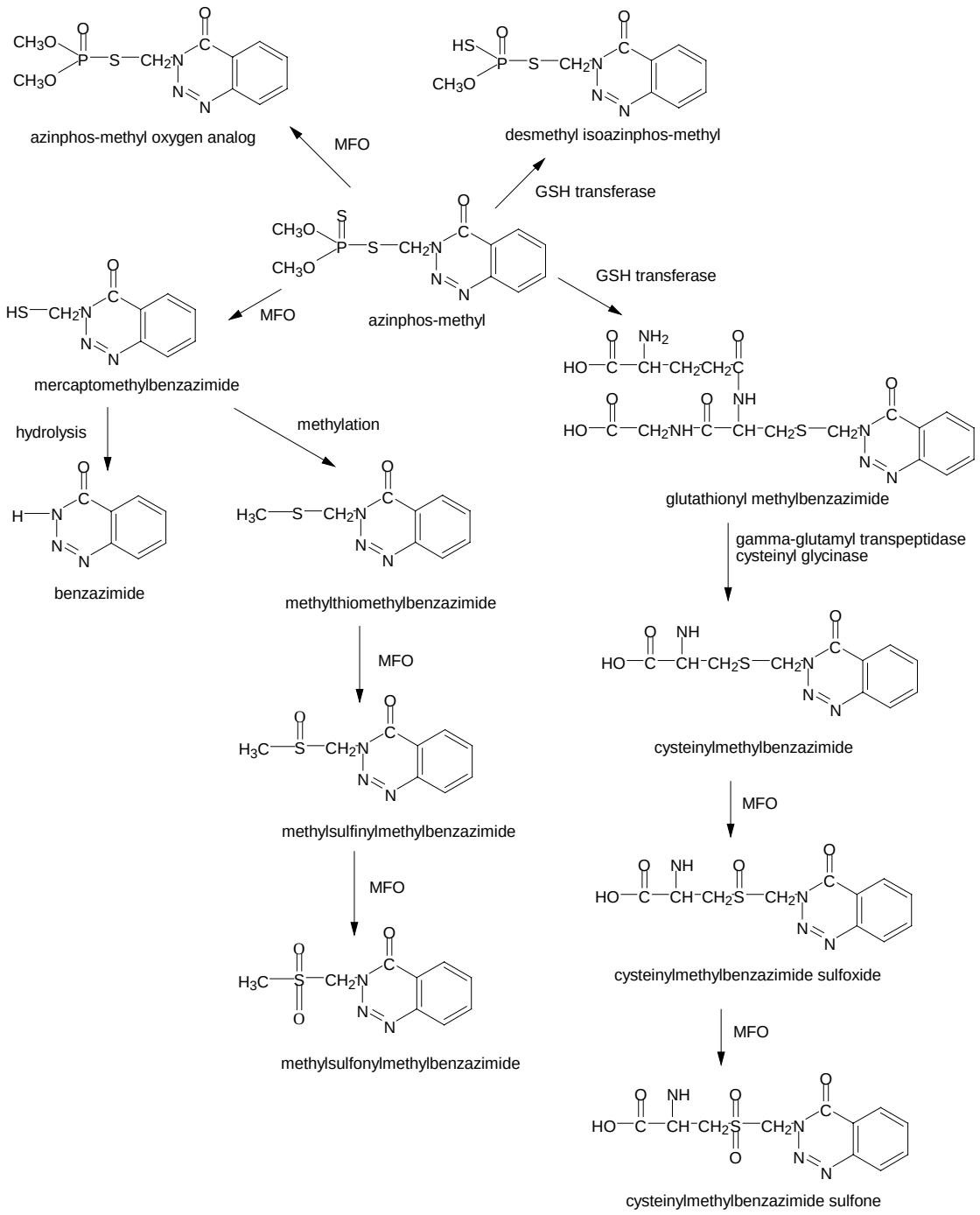

Figure 1. Proposed metabolic pathway for azinphos-methyl in rats (Kao, 1988)

E. Excretion

Within 48 hours after rats and chickens were administered azinphos-methyl by the oral route, more than 90% of the total dose was eliminated in the excreta (Ecker, 1976; Patzschke *et al.*, 1976; Kao, 1988; Scheele *et al.*, 1977). The excretion in cattle was slower with only 52% of the applied dose excreted by 48 hrs, 40% in urine and 12% in feces (Everett *et al.*, 1966). In rats, 60-80% and 15-35% of the total dose was excreted in urine and feces, respectively, irrespective of the route of administration (Ecker, 1976; Kao, 1988). Less than 0.1% was eliminated from the lungs. In lactating cows and goats, less than 1% of the applied dose was excreted in milk (Everett *et al.*, 1977; Gronberg *et al.*, 1988).

The excretion of azinphos-methyl appears to fit a two-compartment model based on its disappearance from tissues in rats (Patzschke *et al.*, 1976). The elimination half-life was approximately 10 hrs for the alpha-phase and 10 days for the beta-phase. The slower elimination phase may be due to the apparent binding of azinphos-methyl and/or its metabolites to hemoglobin.

F. Benzazimide Metabolite

Weber *et al.* (1980) studied the pharmacokinetic behavior of the plant and animal metabolite, benzazimide, in rats. Greater than 95% of benzazimide administered orally was absorbed. More than 99% of the amount administered was excreted in the urine (54-66%) and feces (33-45%) within 48 hours. The elimination half-life for all tissues was approximately 4 days with the slowest elimination in blood and RBCs ($t_{1/2}$ = 11 days). The identification of metabolites, if any, was not attempted.

G. Conclusions

Azinphos-methyl appears to be readily absorbed and rapidly metabolized in the species examined. The oral absorption rate for azinphos-methyl was assumed to 100% based on studies in rats, cattle and chickens. No data were available on the respiratory uptake and absorption of azinphos-methyl; therefore, a default assumption of 100% was used.

IV. ACUTE TOXICITY

A. Introduction

The Department of Pesticide Regulation has in their worker illness database a number of cases associated with possible or probable occupational exposure to azinphos-methyl. In addition, there is one study conducted in human volunteers which established a NOEL for plasma and RBC ChE inhibition. The standard battery of acute toxicity tests (oral and dermal LD_{50} tests, inhalation LC_{50} tests, dermal and ocular irritation tests, and dermal sensitization tests) were available for both the technical grade material and various formulations including wettable powders, liquid concentrates and dusts. In addition, acute toxicity tests were available for two plant and animal metabolites, benzazimide and methyl benzazimide. Acute effects observed in developmental and neurotoxicity studies for azinphos-methyl were not included here, but will be discussed later under those sections. All acute effects for the technical grade material are summarized under Acute Toxicity in the Hazard Identification section of the Risk Analysis.

B. Human Studies

A study was conducted by McFarlane and Freestone (1998) in human volunteers to determine a NOEL for plasma and RBC ChE inhibition after a single exposure to azinphos-methyl. Male human volunteers were administered azinphos-methyl orally in capsules at 0 (lactose), 0.25, 0.5, 0.75 or 1.0 mg/kg and followed for 14 days after dosing. Dose levels were administered to volunteers (7 treated, 3 controls) in an ascending stepwise manner to minimize causing any toxic effects. In addition, 7 females were administered azinphos-methyl at 0.75 mg/kg along with 3 female control subjects. Female subjects were not pregnant and used Aadequate contraceptive precautions." The average age, weight and height of male subjects were 32.7 years, 75.52 kg, and 175.7 cm, respectively. The average age, weight and height of female subjects were 31.0 years, 63.83 kg, and 165.0 cm, respectively.

At 8 and 24 hours after dosing, there was a significant trend for increased plasma ChE activity relative to baseline in male subjects; however, pairwise comparisons with control subjects was not statistically significant at either of these time points at any dose level. Females also had a significant increase in plasma ChE activity relative to controls at 72 hours. There was also a significant trend for increased RBC ChE activity in males relative to baseline at 72 hours. At this time point, only the mean RBC ChE activity in males at 0.25 mg/kg was significantly higher than control subjects by pairwise comparison. A significant increase in RBC ChE activity was also seen in females at 0.75 mg/kg 2 hours after dosing. The toxicological significance of an increase in ChE activity is uncertain and seems unlikely to be treatment-related. A significant reduction in the mean RBC ChE activity (12% relative to baseline) was seen in males at 0.25 mg/kg 12 hours after dosing. However, the toxicological significance of this reduction is uncertain since the mean RBC ChE activity was significantly higher relative to baseline in males at 0.5 and 0.75 mg/kg/day at this time point. The NOELs for plasma and RBC ChE inhibition were 1.0 and 0.75 mg/kg for males and females, respectively, the highest dose levels tested.

In addition to plasma and RBC ChE activity, a variety of other parameters was measured at various time points during the study. These parameters included vital signs, electro-cardiograms, hematology, clinical chemistry and urinalysis. Physical examinations were given prior to dosing and at 72 hours and 14 days after dosing. Besides vital signs, the physical examinations included assessments for respiratory effects, neurological and neuromuscular activity (pupils, ophthalmoscopy, cranial nerves, strength, sensation, reflexes, cerebellar function) cardiac functioning, and any other Aevents.@ None of the measured parameters, physical signs or clinical observations gave any indication of clinically significant or compound-related effects. Some Aadverse events@ (headaches, runny noses, blurred vision, diarrhea, etc.) were noted at all dose levels including controls, but there were no trends and they did not appear to be treatment-related, especially in absence of any ChE inhibition. Volunteers were not subjected to any neurobehavioral or neurophysiological testing to evaluate for more subtle neurological effects in cognition or nerve conduction. However, given that no significant plasma or RBC ChE inhibition was seen, no neurological effects would be anticipated based on the available neurotoxicity data for azinphos-methyl in animals. DPR has no requirement for human testing of pesticides and there are no FIFRA guidelines for this type of study. However, the study was conducted in a double-blind manner following AGood Clinical Practices@ guidelines and had an extensive informed consent form. Subjects were free to leave the study at any time and were paid in full if they left for health reasons.

An epidemiology study in which a cohort of 90 male apple orchard applicators from New York State were evaluated to determine if short-term exposure to azinphos-methyl produced acute health effects (Stokes et al., 1995). The applicators were first questioned off season and then again during the spraying season for the presence of several acute signs and symptoms. Short-term exposure was validated by measuring dimethylthiophosphate in the urine. Chronic signs of peripheral nerve damage were determined by vibration sensitivity thresholds in both hands and feet during the off season. Long-term exposure to pesticides was determined by questionnaire. Seventy-eight applicators (86%) had used azinphos-methyl during the previous two growing seasons. The mean number of years azinphos-methyl had been used by the applicators was 14 years. The average number of applications per season was 5 times. Of the acute signs and symptoms related to organophosphate poisoning, only headaches were more frequent during the spraying season than off. The mean vibration threshold scores for the hands were significantly higher for applicators when compared with scores for the population based controls matched on age, sex, and county of residence.

Several studies were available in the literature in which plasma and/or RBC ChE activities were monitored in orchard applicators or harvesters exposed to azinphos-methyl. Sixteen thinners, 3 foremen, and 2 irrigators were evaluated over a 5-day period for whole blood ChE activity and urinary dialkylphosphate levels after working in peach orchards treated 14 days prior with azinphos-methyl at 2 lb a.i./acre (Kraus *et al.*, 1977). Workers were also given pre- and post-exposure physical examinations in which they were evaluated for symptoms of organophosphate poisoning, with particular emphasis on reflex activity. A significant reduction in whole blood ChE activity to 85.2% of baseline was observed in the thinners from the first to fifth day of exposure. Dimethylthiophosphate was detected in the urine of all the thinners during exposure, while the foremen and irrigators contained only very small quantities of this

metabolite. It was more difficult to obtain reflex action in the upper extremities of 13 of the 21 workers during post-exposure examination compared to the pre-exposure examination. No effect on lower extremity reflexes was seen. The one thinner with the greatest reduction in whole ChE activity (-29.8% on Day 5), lost 2.5 kg.

The same group of investigators monitored plasma and RBC ChE activity and urinary dialkylphosphate levels in another 15 male peach thinners a year later (Richards *et al.*, 1978). Eight males were assigned to a plot treated with azinphos-methyl at 2.5 lb a.i./acre and the other 7 were assigned to a plot treated with the pesticide, Galecron which does not inhibit ChE. The peaches were treated with azinphos-methyl 14 days prior to the 5-day exposure period. A significant decrease of less than 10% was seen in both groups of men relative to their baseline activity. When compared to each other only the RBC ChE activity was significantly reduced in azinphos-methyl exposed workers compared to controls on Day 5 (−8.3% vs. −3.8% of baseline). The plasma ChE activity in azinphos-methyl exposed workers was not significantly different from the control workers at any time point. The mean urinary dimethylphosphate and dimethylthiophosphate levels correlated with the mean percent decline in RBC ChE activity from baseline ($r = -0.663$ and -0.874, respectively). No symptoms related to organophosphate toxicity were reported by the workers during or after exposure.

Franklin *et al.* (1981) measured urinary alkyl phosphates and blood ChE activity in 14 mixer/loader/applicators exposed to azinphos-methyl during its application to orchards. The orchards were sprayed using ultra-low volume procedures with airblast sprayers at 1.25 lb of a 50% azinphos-methyl wettable powder formulation per acre. Workers sprayed for only 1 day. Reductions in serum and RBC ChE activity were less than 5% on the day of exposure. Urinary alkyl phosphates were detected during the 48 hours following spraying. The level of urinary metabolites showed a weak to moderate correlation ($r = 0.48$, 24-h; $r = 0.77$, 48-h) with the amount applied, but only a weak correlation with the time sprayed ($r = 0.43$, 24-h & 48-h). No attempt was made to correlate urinary alkyl phosphate levels with the serum or RBC ChE activity.

Ninety-seven agricultural workers (71 men, 26 women) exposed to methidathion, vamidothion, and azinphos-methyl sprayed in orchards over two growing seasons were monitored for urinary dialkylphoshates and serum ChE activity (Drevenkar *et al.*, 1991). Paraoxonase and arylesterase activities in the serum were also measured. The workers consisted of 20 mixers, 42 sprayers, 23 field workers (cutters), and 12 people with no direct contact with the pesticides (managers, mechanics, a technologist and a housekeeper). Methidathion and vamidothion were applied during the first growing season and azinphos-methyl during the second growing season. Blood and urine samples were collected one month before the beginning of the first spraying season and about three months later for the first growing season. For the second growing season, blood and urine sample were collected only after a 2-day spraying session. More than one dialkylphosphorus metabolite was detected in the urine of most after-exposure urine samples. The highest concentrations were found after exposure to azinphos-methyl. The after-exposure serum ChE activities were reduced from 11 to 30% from baseline in 26 workers and 31-48% from baseline in 12 workers (6 sprayers, 3 field workers, 2 mixers and 1 mechanic). However, 4 of the 12 workers with ChE inhibition greater than 30% had no urinary

dialkylphosphates. No correlation between the ChE activities and urinary metabolites was observed. None of these 12 workers had any complaints that were attributed to organophosphate poisoning. Paraoxonase and arylesterase activities were unaffected.

Urinary alkylphosphate and blood ChE activities were monitored in 33 peach harvest workers (pickers and sorters) in California (Schneider *et al.*, 1994). The pickers served as the exposed group and the sorters as the control or minimally exposed group. The orchard had been sprayed with azinphos-methyl once at 1.5 lb a.i./acre 51 days before harvesting began. Baseline ChE measurements were taken one week prior to the initial exposure. No significant difference in the plasma ChE activity between the exposed and control groups was seen on either day 14 or 23 of exposure. However, the RBC ChE was significantly reduced (77-87% of control activity) on both days 14 and 23 of exposure. There was a significant inverse correlation ($r = -58$ to -65) of the RBC ChE activity and the urinary alkylphosphate levels. Although there was also an inverse correlation ($r = -21$ to -37) between the plasma ChE activity and urinary alkylphosphate levels, the correlation was not significant

In a study conducted by McCurdy et al. (1994) the urinary alkylphosphate metabolites, plasma and RBC ChE activities and their reactivation after incubation with 2-aldoxime methochloride (2-PAM) were evaluated in 20 peach harvest workers in California. The harvesters worked 21 days over a 6-week period in an orchard that had been sprayed with azinphos-methyl (1.5 lb a.i./acre) 30 days previously. The median RBC ChE activity for all workers decreased 7% from baseline during an initial 3-day period and 19% from baseline over the 6-week period. The median plasma ChE activity decreased 9% during the initial 3-day and 12% over the 6-week period. However, no subjects had a positive oxime reactivation test. The workers had urinary azinphos-methyl metabolites (dimethylphosphate, dimethylthio-phosphate, and dimethyldithiophosphate) which increased steadily during the 6-week exposure period. There was a poor correlation between plasma ChE activity and the urinary metabolites ($r = 0.09$ and -0.39 on days 3 and 44, respectively), but there was a better correlation with RBC ChE activity and exposure ($r = -0.77$ and -0.51 on days 3 and 44, respectively). The only evaluation for other health effects was a questionnaire that addressed general health.

Carrier and Brunet (1999) applied a toxicokinetic model to the data from the study conducted by McCurdy et al. (1994) to estimate a No-Observed-Adverse-Effect Level (NOAEL). They considered the RBC ChE inhibition observed in this study to not be adverse since no symptoms or signs were observed; therefore, the exposure level in these workers was considered a NOAEL. They assumed the dermal absorption of azinphos-methyl in humans was 16.1% based on the study by Feldman and Maibach (1974). They also used urinary metabolite data after intravenous injection from the Feldman and Maibach (1974) study to estimate a half-life for azinphos-methyl of 32.6 hrs. They estimated the absorbed NOAEL for a single exposure to be 0.3 mg/kg. This would be equivalent to an external dose of 1.9 mg/kg. They estimated the absorbed NOAEL for repeated exposure to be 0.1 mg/kg/day. This is equivalent to an external dose of 0.62 mg/kg/day.

Illnesses or injuries associated with exposure to azinphos-methyl alone or in combination with other pesticides were described in Part B (Exposure Assessment) of the Evaluation of

Azinphos-methyl as a Toxic Air Contaminant and are only briefly described here. In California, DPR has records for 156 illnesses/injuries associated with azinphos-methyl between 1984 and 1996 (Formoli, 1999). At least 75% of these cases involved occupational exposure and more than 80% of the illnesses were systemic. Most of the illnesses were due to a few incidents where a number of workers were exposed, including one incident in 1987 involving 37 peach harvesters and another in 1993 involving 14 almond pruners. Most of the non-occupational illnesses also occurred in clusters, one in 1987 involving 26 cases and another in 1993 involving 8 cases. In both cases azinphos-methyl drifted into nearby residential areas. In the 1987 drift incident, the weather was reported to be hot, with a temperature inversion and no wind (Mehler, 2000). Symptoms reported with this drift incident included burning eyes, skin, mouth and lungs, sore or raspy throat, nasal discharge, nose bleed, blurred vision, headache, salivation, nausea, vomiting, abdominal cramps, diarrhea, dizziness, sweating, chest pain, and difficulty breathing. A temperature inversion was not reported with the 1993 drift incident, but apparently there was a noticeable pesticide odor. Swab samples were negative for pesticides. Symptoms reported with this incident included shortness of breath, coughing, difficulty breathing, headache, sore throat, burning nasal passages, stuffy nose, vomiting, and dizziness. None of these cases necessitated hospitalization or time off work.

C. Animal Studies

1. Systemic Effects

Acute toxicity of azinphos-methyl varies depending on species, sex, route, and formulation (Table 1-3). In rats, females tended to be more sensitive than males for all routes of exposure. It is less clear if there were sex differences for other species. The acute inhalation toxicity of azinphos-methyl is summarized in Table 1. The 1-hour LC_{50} values for the technical grade material were within an order magnitude (38 to 385 mg/m^3) except in one study which reported an LC_{50} greater than 17,560 mg/m^3 after a 1-hour, whole body exposure (Harris, 1976a). This study had several major deficiencies including no analysis of test article or particle size, inadequate description of test conditions and results, and inadequate number of dose levels. In a 4-hour inhalation study (head-only), all of the female rats at the lowest dose tested (80 mg/m^3 or 14.4 mg/kg)[1] exhibited several cholinergic signs (ocular and nasal discharge, salivation, hypoactivity, tremors, and/or twitching) (Shiotsuka, 1987). This study was acceptable to DPR toxicologists based on the Federal Insecticide, Rodenticide, and Fungicide Act (FIFRA) guidelines. No mortalities occurred at this dosage. Red turbinates and lungs were observed at necropsy in several high-dose animals that died. An acute inhalation NOEL of 23 mg/m^3 (4.1 mg/kg)[2] was established in male rats exposed (whole body) for 4 hours to azinphos-methyl

[1] The mass mean aerodynamic diameters ranged from 1.6 to 2.0 μm; therefore, 100% respiratory uptake and absorption was assumed. In converting to mg/kg, assumed a female Sprague-Dawley rat weighed 204 kg and breathed 0.037 m^3 in 4 hours (U.S. EPA, 1988).

[2] No data on particle size was available; however, a default assumption of 100% respiratory uptake and absorption was used. In converting to mg/kg, assumed a male Wistar rat weighed 215 g and breathed 0.0383 m^3 in 4 hours (U.S. EPA, 1988).

Table 1. Summary of Acute Inhalation Toxicity for Azinphos-methyl

Species	Gender	LC_{50} (mg/m^3)	References[a]
		Technical Grade (86 - 90%)	
Rat	M	385 (1-hr, whole body)	1
	F	107 (1-hr, whole body)	2
	M/F	>17,560 (1-hr, whole body)	3
	M	152 (4-hr, whole body)	1
	M	155 (4 hr, head only)	4*
	F	132 (4-hr, head only)	
Mouse	F	38 (1-hr, whole body)	2
		Wettable Powders (25-62.5%)	
Rat	M	200 - >5,000 (1-hr, whole body)	5-7
	F	169 - 4,000 (1-hr, whole body)	5-8
	M/F	>17,560 (1-hr, whole body)	9
	M	198 - 596 (4-hr, head or nose only)	7,10
	F	170 - 422 (4-hr, head or nose only)	7,10
		Liquid Concentrates (12.1-24%)	
Rat	F	475 (30-min, whole body)	11
	M	820 - 3,000 (1-hr, whole body)	12-16
	F	590 - >2,600 (1-hr, whole body)	12-16
Mouse	F	190 (1-hr, whole body)	11
	M	<2,000 (1-hr, whole body)	12
		Dust (2%)	
Rat	F	>20,000 (1-hr, whole body)	17
Mouse	F	>20,000 (1-hr, whole body)	

a References: 1. Kimmerle, 1966; 2. Doull and DuBois, 1956; 3. Harris, 1976a; 4. Shiotsuka, 1987; 5. Crawford and Anderson, 1970; 6. Cannon and Taylor, 1978; 7. Shiotsuka, 1986; 8. Nelson and Doull, 1967; 9. Harris, 1976b; 10. Warren, 1990; 11. DuBois, 1967; 12. DuBois and Kleeburg, 1970; DuBois and Kinoshita, 1970; 14. DuBois, 1970b; 15. Nelson, 1978c; 16. Cannon and Taylor, 1979; 17. Crawford and Nelson, 1970b.

* Acceptable study based on FIFRA guidelines

(Kimmerle, 1966). All of the males at the LOEL (59 mg/m^3) exhibited unspecified signs of toxicity. This study also had several major deficiencies including no analysis of test article or particle size and inadequate description of test conditions and results. The one-hour LC_{50} values for formulations varied from 245 mg/m^3 in female rats exposed (head only) to a 50% wettable powder (Shiotsuka, 1986) to greater than 20,000 mg/m^3 in female rats and mice exposed (whole body) to a 2% dust (Crawford and Nelson, 1970b).

By the oral route, rats and dogs appear to be more susceptible to the acute toxicity of azinphos-methyl than guinea pigs (Table 2). The oral LD_{50} values for technical grade azinphos-methyl ranged from 4.4 mg/kg to 26 mg/kg for rats. The clinical signs observed with the technical grade material included tremors, twitching, convulsions, staggering gait, prostration, salivation, breathing difficulties, lethargy, and piloerection, all typical of ChE inhibition. The

Table 2. Summary of Acute Oral Toxicity for Azinphos-methyl

Species	Gender	LD_{50} (mg/kg)	References[a]
		Technical Grade (88.9 - 99.0%)	
Rat	M	4.6 - 26	1-7
	F	4.4 - 24	2-9
Guinea pig	M	80	8
Dog	M	10	6
		Wettable Powders (35-62.5%)	
Rat	M	23.6 - 58	10-13
	F	14.8 - 58	10-14
		Liquid Concentrates (12.1-24%)	
Rat	M	37 - 101	15-19
	F	21 - 85	18-23
	M/F	37	24
Mouse		NR[b]	25
		Dusts (2%)	
Rat	F	>50	26

a References: 1. Hecht, 1955; 2. Gaines, 1960; 3. Crawford and Anderson, 1974; 4. Lamb and Anderson, 1974; 5. Pasquet *et al.*, 1976; 6. Mihail and Lorke, 1978; 7. Heimann, 1982; 8. DuBois *et al.*, 1957a; 9. Nelson, 1968; 10. DuBois, 1970a; 11. Cooper *et al.*, 1978; 12. Nelson, 1979b; 13. Sheets, 1990a; 14. Bauman and Nelson, 1969; 15. DuBois, 1962a; 16. DuBois and Kinoshita, 1965c; 17. DuBois and Kinoshita, 1970; 18. Nelson, 1978a; 19. Nelson, 1979a; 20. DuBois, 1963; 21. Nelson and Bauman, 1968; 22. Nelson and Bauman, 1969; 23. DuBois, 1970b; 24. Lightowler and Gardner, 1978a; 25. Sato, 1959; 26. Crawford and Nelson, 1970a.

b NR = Not Reported

onset of signs was 5 to 20 minutes after dosing and usually lasted 1-2 days. There were no compound-related abnormalities observed in the one study that reported necropsy findings (Mihail and Lorke, 1978). A NOEL could not be established in most studies either due to the dose levels being too high or insufficient information, but in one study a NOEL was established for rats at 1 mg/kg/day (Mihail and Lorke, 1978). All of the animals (males and females) at the LOEL (2.5 mg/kg) exhibited unspecified cholinergic signs. The oral LD_{50}'s for formulations ranged from 14.8-101 mg/kg depending on the percent active ingredient and species. In addition to the clinical signs observed with the technical grade material, lacrimation, exophthalmos, clear and red nasal discharge, anorexia, vomiting, diarrhea, perianal stains, and alopecia were also observed. These signs are typical of ChE inhibitors and are probably due to the active ingredient.

The acute dermal toxicity of technical grade azinphos-methyl and various formulations is summarized in Table 3. The LD_{50} values for the technical grade material were similar (72-250 mg/kg) except for one study which reported an LD_{50} of 2,500 to 5,000 (Mihail and Lorke, 1978). The clinical signs observed were similar to those observed with the oral route, except that erythema was noted at the site of application. A NOEL was not established for the technical grade material in any of the studies. A LOEL of 63 mg/kg in female rats was reported (Heimann, 1982). There were no mortalities at the LOEL, but all females at the LOEL exhibited unspecified

Table 3. Summary of Acute Dermal Toxicity for Azinphos-methyl

Species	Gender	LD_{50} (mg/kg)	References[a]
		Technical Grade (88.9 - 99.0%)	
Rat	M	200 - 5,000	1-4
	F	72 - 5,000	1,3-5
		Wettable Powders (35-62.5%)	
Rat	M	816 - >2,000	6-8
	F	300 - >2,000	7-9
Rabbit	M	1,137	10
	F	1,147	
	M/F	1,780	11
		Liquid Concentrates (12.1-25%)	
Rat	M	322 - 475	12-13
	F	150 - >1,500	14-17
	M/F	325	18
Mouse	NR[b]	65	19
Rabbit	M	504 - >1,500	14,20
	F	568	20
		Dusts (2%)	
Rat	F	>2,000 mg/kg	21

a References: 1. Gaines, 1960; 2. Pasquet *et al.*, 1976; 3. Mihail and Lorke, 1978; 4. Heimann, 1982; 5. Nelson, 1968; 6. DuBois and Kinoshita, 1970; 7. Sheets, 1990b; 8. DuBois, 1970a; 9. Nelson, 1967a; 10. Nelson, 1979c; 11. Seaman and Imlay, 1978; 12. DuBois and Murphy, 1956; 13. DuBois and Kinoshita, 1965c; 14. DuBois, 1963; 15. Nelson, 1967b; 16. Nelson and Bauman, 1968.; 17. Nelson and Bauman, 1969; 18. Lightowler and Gardner, 1978b; 19. Sato, 1959; 20. Nelson, 1978b; 21. Crawford and Nelson, 1970a.

b NR = Not Reported

cholinergic signs. Possible compound-related gross lesions observed at necropsy in these studies were pulmonary emphysema, enlarged adrenal glands, dark liver, pale spleen, reddened renal medulla, and ulcers (Mihail and Lorke, 1978; Heimann, 1982). The LD_{50} values for the formulations varied from 65 mg/kg in mice exposed to a 20% emulsifiable concentrate (Sato, 1959) to greater than 2,000 mg/kg in rats exposed to a 2% dust (Crawford and Nelson, 1970a) or a 35% wettable powder (Sheets, 1990b).

There are several reports of biochemical/histochemical changes in the liver after a single dose of azinphos-methyl. The effect of azinphos-methyl on liver glycogen is unclear. Murphy and Porter (1966) reported that liver glycogen levels increased 8 to 15-fold in rats after an intraperitoneal injection of azinphos-methyl at 3 mg/kg. El-Banhawy and El-Ganzuri (1986) reported marked depletion of liver glycogen in rats administered a single dose of azinphos-methyl orally at 6.5 mg/kg. The glycogen depletion in this study was based on the loss of glycogen inclusions in liver cells examined histologically. One explanation for the different findings may be the difference in the time at which the animals were sacrificed. El-Banhawy and El-Ganzuri sacrificed their animals 24 hrs after dosing whereas Murphy and Porter sacrificed

their animals 5 hrs after dosing. El-Banhawy and El-Ganzuri (1986) also reported a disintegration and subsequent loss of lipoid inclusions in liver cells of rats given a single dose of azinphos-methyl at 6.5 mg/kg. Murphy and Porter (1966) reported an increase in liver alkaline phosphatase and tyrosinetransaminase activities in the rats given a single dose of azinphos-methyl at 3 mg/kg. The toxicological significance of these findings is uncertain.

2. Local Effect

Technical grade azinphos-methyl caused only slight conjunctival redness in rabbits that cleared by 48 hrs (Table 4). The various formulations were more severe ocular irritants causing slight to severe conjunctival redness, very slight to moderate chemosis, slight to severe ocular discharge, slight to moderate corneal opacity, and slight iritis which cleared by day 7.

Table 4. Summary of Eye Irritation Potential of Azinphos-methyl

Species	Gender	Results	References[a]
		Technical Grade (~92%)	
Rabbit	M/F	Slight Irritation	1-2
		Wettable Powders (25-50%)	
Rabbit	M/F	Slight-Moderate Irritation	3-6
		Liquid Concentrates (22%)	
Rabbit	M/F	Slight-Moderate Irritation	7-8

a References: 1. Thyssen, 1981; 2. Harris, 1976a; 3. Hixson, 1979; 4. Sheets, 1990c; 5. Seaman, 1978a; 6. Harris, 1976b; 7. Nelson, 1978d; 8. Knapp and Doyle, 1979a.

No dermal irritation was observed in rabbits exposed to technical grade azinphos-methyl; however, slight erythema was observed in humans after a 24-hour exposure (Table 5). The inert ingredients appear to be responsible for the dermal irritation (slight to moderate erythema and very slight to slight edema) observed with several formulations.

Table 5. Summary of Dermal Irritation Potential of Azinphos-methyl

Species	Gender	Results	References[a]
		Technical Grade (~92%)	
Rabbits	M/F	No irritation	1-2
Humans	NR[b]	Slight Irritation	3
		Wettable Powder (25-50%)	
Rabbits	M/F	No to Slight Irritation	4-7
		Liquid Concentrates (22%)	
Rabbits	M/F	Slight Irritation	8-9

a References: 1. Thyssen, 1981; 2. Harris, 1976a; 3. Hecht, 1955; 4. Hixson, 1979; 5. Sheets, 1990d; 6. Seaman, 1978b; 7. Harris, 1976b; 8. Nelson, 1978d; 9. Knapp and Doyle, 1979b.

b NR = Not Reported

Technical grade azinphos-methyl appears to be a weak to moderate dermal sensitizer using the Buehler's patch test (Table 6). The sensitization response was variable with the formulations being the same or weaker than the technical grade material. In a modified Buehler's patch test, a 12.5% solution of azinphos-methyl was applied topically to male guinea pigs once a week for 3 weeks during the induction phase (Heiman, 1987). Two weeks later, they were challenged with a 6% solution. Six of 12 animals tested reacted positively to the challenge. Two weeks following the first challenge, the same animals were challenged a second time with a 0.6% solution. None of the animals reacted to the second challenge. This finding suggests that there may be a threshold for this response. The time between exposures may be another factor.

Table 6. Summary of Dermal Sensitization Potential of Azinphos-methyl

Species	Gender	Results	References[a]
		Technical Grade (89-92%)	
Guinea Pig	M	Weak to Moderate Sensitization	1-2
		Wettable Powders (35-50%)	
Guinea Pig	M	No to Moderate Sensitization	3-4
		Liquid Concentrates (22%)	
Guinea Pig	M	No Sensitization	5

a References: 1. Porter *et al.*, 1987a; 2. Heimann, 1987; 3. Rosenfeld, 1984a; 4. Porter *et al.*, 1987b; 5. Rosenfeld, 1984b.

3. Metabolites - Benzazimide and Methyl Benzazimide

The acute toxicity of two metabolites of azinphos-methyl, benzazimide and methyl benzazimide, was evaluated (Crawford and Anderson, 1974; Lamb and Anderson, 1974). These metabolites are common in both plants and animals. The oral LD_{50} values for benzazimide ranged from 269 to 576 mg/kg in rats with females being slightly more susceptible than males. The oral LD_{50} for methyl benzazimide ranged from 330 to 524 mg/kg in rats with males and females being equally sensitive. The clinical signs observed with both metabolites were sedative in nature, including lethargy, sedation, dyspnea, and comatose. These signs and death were observed at doses as low as 200 mg/kg of benzazimide in female rats. The LOEL for methyl benzazimide was 250 mg/kg. A NOEL was not established for either benzazimide or methyl benzazimide.

4. Synergism

Synergism is sometimes observed when two organophosphate chemicals are given simultaneously. The combined acute toxicity of azinphos-methyl and certain organophosphates was additive, including EPN, methyl parathion, methiocarb, fenitrothion, and trichloronate (DuBois, 1956a; DuBois *et al.*, 1957b; DuBois and Raymund, 1961; DuBois and Kinoshita, 1963a & 1965a). The acute toxicity was less than additive when azinphos-methyl was combined with other organophosphates, such as malathion, demeton, parathion, fensulfothion and naftalofos (DuBois, 1956b&c; DuBois and Kinoshita, 1963b and 1965b). DuBois (1956c)

suggested that the less than additive response was due to significantly different rates in the conversion of the chemicals to the active metabolite or the detoxification resulting in different times of peak cholinesterase inhibition. Evidence of a synergistic effect were found with several other organophosphates and azinphos-methyl, including ethion, crufomate, and trichlorfon (DuBois, 1962b; DuBois, 1958; McCollister *et al.*, 1968). For these combinations, the acute toxicity was 1.5 to 2.2 times greater than expected if due to simple additivity. There was also evidence of synergism with another study in which azinphos-methyl was tested in combination with 21 other chemicals (Witherup and Schlecht, 1963). Interpretation of the findings from this finding was more difficult since the chemicals were only tested in combination at the LD_{01} level. Factorial analysis was used to determine if there were significant interactions between the chemicals. Seven chemicals, coumaphos, crotoxyphos, DDVP, diazinon, dicrotophos, disulfoton and ronnel, had significant interactions with azinphos-methyl indicating synergism. It was not possible with this method of analysis to determine the degree of synergism other than the level of significance. It was also not possible to determine if the interaction between the other chemicals (carbaryl, demeton, dimethoate, dioxathion, EPN, ethion, malathion, methyl parathion, mevinphos, OPMA, naled, parathion, phosphamidon, and trithion) was additive or less than additive.

Pretreatment with diethyl maleate, which depletes glutathione levels by conjugating with glutathione, enhanced the acute toxicity of azinphos-methyl in mice (Sultatos and Woods, 1988). On the other hand, these same investigators found that buthionine sulfoximine, a selective inhibitor of glutathione synthesis, did not affect the acute toxicity of azinphos-methyl. They concluded that glutathione conjugation is of minor importance in the detoxification of azinphos-methyl because these two chemicals had different effects on the acute toxicity. The investigators suggested that diethyl maleate may be enhancing the acute toxicity of azinphos-methyl through some other metabolic pathway.

D. Conclusions

No clinical signs, plasma or RBC ChE inhibition were observed in one human study where males and females received up to 1.0 and 0.75 mg/kg, respectively. ChE activity was not measured in any of the acute toxicity tests in laboratory animals. The signs observed in laboratory animals after acute exposure to azinphos-methyl were typical cholinergic signs (tremors, twitching, salivation, convulsions, and breathing difficulties). Red turbinates and lungs were observed at necropsy in animals that died after inhalation exposure. Erythema was observed with dermal exposure. The plant and animal metabolites, benzazimide and methyl benzazimide, produced clinical signs that were sedative in nature. These metabolites appear to be less lethal than azinphos-methyl based on their oral LD50 values which were at least an order of magnitude higher. A NOEL was not established in most of the acute toxicity studies due to the high dosages used in these studies. However, a NOEL of 23 mg/m^3 (4.1 mg/kg) was observed in one 4-hour (whole body) inhalation study in male rats based on unspecified signs of toxicity.

V. SUBCHRONIC TOXICITY

A. Introduction

Four subchronic toxicity studies in laboratory animals were available for azinphos-methyl, including one inhalation study, two oral studies and one dermal study. A 25% wettable powder was used in both oral studies. The technical grade material was used in the inhalation and dermal study. The exposure period was 3 to 4 months in all the studies except the dermal study. Rats were used in all but the dermal study. The dermal study was conducted in rabbits and had an exposure period of 3 weeks. None of the animal studies met the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines. A 28-day oral study with human volunteers was also available. Although there are no FIFRA guidelines for human studies, this study had major deficiencies.

B. Human Studies

Five male human volunteers/dose were given azinphos-methyl in capsules (corn oil vehicle) at doses between 1 and 20 mg/day (14 to 286 µg/kg/day for 70 kg person) for 30 days (Rider *et al.*, 1972). ChE activity was measured twice weekly during the exposure period. No plasma ChE inhibition was observed at doses up to 20 mg/day. No RBC ChE inhibition was seen at doses up to 18 mg/day, but erratic inhibition was seen at 20 mg/day. However, the investigators did not consider the RBC ChE inhibition at 20 mg/day sufficient to be considered an adverse effect. There was also no effect on clinical signs, hematology, prothrombin time, and urinalysis. Therefore, the NOEL was determined to be greater than or equal to 20 mg/day (286 µg/kg/day) based on plasma and RBC ChE inhibition. Although there are no FIFRA guidelines for conducting human studies, this study had several obvious deficiencies (insufficient information including no summary tables or individual data and inadequate exposure period).

Karr *et al.* (1992) evaluated the dermal exposure and monitored the plasma and RBC ChE activity in 48 apple orchard applicators. All applicators, except for one, sprayed 50 or 35% wettable powder azinphos-methyl alone or in combination with 78% liquid phosphamidon. Forty slaughterhouse workers served as controls. Baseline ChE levels were measured prior to the first spraying of the season. The postseason levels were measured at least 3 weeks after the last pesticide application of the season. There was a mean postseason reduction in RBC ChE activity of –3.9%. Among applicators that sprayed more than 10 days during the season, the mean RBC ChE activity was even lower (–5.7%). For six applicators that had quantitative dermal exposure estimates, the changes in RBC ChE activity correlated with the estimated dermal exposure ($r = -0.84$). There was no reduction in the mean postseason plasma ChE activity nor was there any correlation in the plasma ChE activity and estimated dermal exposure.

B. Animal Studies

1. Inhalation - Rats

Ten SPF Wistar rats/sex/dose were exposed (whole body) to technical grade azinphos-methyl (purity not reported) at 0, 0.195, 1.24 or 4.72 mg/m^3 (0, 0.05, 0.32 or 1.26 mg/kg/day)[3] for 6 hrs/day, 5 days/wk for 12 weeks (Kimmerle, 1976). There was no effect on appearance, behavior, clinical chemistry, hematology, organ weights, and gross pathological or histological findings. The mean body weights were reduced slightly (~8%) in males at 4.72 mg/m^3. At the study termination, the mean plasma ChE was reduced at 4.72 mg/m^3 (M: 84%; F: 85% of controls activity). The RBC ChE activity was also reduced at 4.72 mg/m^3 (M: 56%; F: 63% of control activity) at the study termination. There was no effect on brain ChE activity in either sex. In general, DPR considers brain ChE inhibition to be indicative of overt toxicity since it is one of the primary target sites and more subtle neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC ChE inhibition is less certain because the ChEs in blood have no known physiological function. Based on the lack of significant findings, the NOEL for overt toxicity was greater than or equal to 4.72 mg/m^3 (1.26 mg/kg/day), the highest dose tested. The NOEL for plasma and RBC ChE inhibition was 1.24 mg/m^3 (0.32 mg/kg/day). This study was unacceptable based on several major deficiencies including no analysis of test article, incomplete clinical chemistry and histopathological examination and no individual data.

2 Oral - Rats

Thirteen Sprague-Dawley rats/sex/dose were fed azinphos-methyl (25% wettable powder) in the diet at 0, 2, 5, or 20 ppm active ingredient (0, 0.2, 0.5 or 1.9 mg/kg/day)[4] for 16 weeks (Doull and Rehfuss, 1956). There was no effect on food consumption or gross and microscopic lesions. Male rats receiving 20 ppm had up to 20% reduction in weight gain. After 16 weeks of treatment at 20 ppm, there was a reduction in the mean ChE activity in the brain (M: 91%, F: 86% of controls), serum (M: 64%, F: 76% of controls), and red blood cells (M: 60%, F: 62% of controls). No ChE inhibition was observed in the 2 ppm or 5 ppm groups. Recovery of the ChE activity was observed in serum, brain and RBCs by 4, 10, and 20 days after the treatment was discontinued. The NOEL was 5 ppm (0.5 mg/kg/day) based on serum, RBC, and brain ChE inhibition and reduced weight gain. This study had major deficiencies including no analysis of the test article or diet, no hematology, no individual data and incomplete clinical chemistry and histopathology.

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[3] Ninety-seven percent of the particles were found to have a diameter of 1 ± 0.5 μ; therefore, it was assumed the respiratory uptake and absorption was 100%. Using the average body weight from the study and assuming a Wistar rat breaths 0.05 m^3 in 6 hours (U.S. EPA, 1988).

[4] Estimated assuming a 235 g Sprague Dawley rat consumes 22 g of feed per day (U.S. EPA, 1988).

In a subsequent study, 18 male Sprague-Dawley rats/dose were fed azinphos-methyl (25% wettable powder) in the diet at 0, 50 or 100 ppm active ingredient (0, 4.7 or 9.4 mg/kg/day)[5] for 16 weeks (Doull and Anido, 1957b). Marked symptoms of cholinergic stimulation including diarrhea, salivation, lacrimation, and muscular fasciculations were observed at both 50 and 100 ppm during the first 4 weeks of exposure (time of onset not reported). There were 8 and 10 deaths at 50 and 100 ppm, respectively. The first death occurred during week 4 at 100 ppm and week 6 at 50 ppm. A decrease in the mean weight gain (10-18%) was observed in both treatment groups. At 50 and 100 ppm, there was a reduction in the mean ChE activity in the plasma (61% and 37% of controls, respectively), RBCs (29 and-27% of controls, respectively) and brain (52 and 25% of controls, respectively). There were no treatment-related changes in the macroscopic and microscopic findings. The LOEL for this study was 50 ppm (4.7 mg/kg/day) based on the cholinergic signs, reduced weight gain, and plasma, RBC and brain ChE inhibition. A NOEL was not established in this study. This study was unacceptable due to major deficiencies (no females, no analysis of the test article or diet, no hematology, no individual data, and incomplete clinical chemistry and histopathology).

3. Dermal - Rabbits

Azinphos-methyl (94.1% purity) was applied with a Cremophor EL and water vehicle to the shaved backs and flanks of 6 New Zealand rabbits/sex/dose at 0, 2 or 20 mg/kg and left uncovered in place for 6 hrs/day, 5 days/wk for 3 weeks (Flucke and Schilde, 1980). An additional 3 rabbits/sex/dose had their skin abraded before being exposed. No significant differences in clinical signs, body weights, clinical chemistry, hematology, urinalysis, organ weights, gross pathological or histological findings (including local effects) were found. A slight to moderate reduction in the mean RBC ChE activity (M - abraded: 62%, M – intact: 77%, F – abraded: 74%, F – intact: 68% of control activity) was seen at 20 mg/kg/day at study termination. There was no effect on plasma or brain ChE activity. The NOEL for overt toxicity was greater than or equal to 20 mg/kg, the highest dose tested. The NOEL for RBC ChE inhibition was 2 mg/kg. This study had several major deficiencies, including too few dose levels and no overt toxicity at the highest dose, and incomplete individual data.

E. Conclusions

The primary effect observed in the subchronic toxicity studies for azinphos-methyl was ChE inhibition. In several studies, reduced body weights were seen. Cholinergic signs (diarrhea, salivation, lacrimation and muscular fasciculations) were observed in one study. In general, DPR considers brain ChE inhibition to be indicative of overt toxicity since it is the primary target site and more subtle neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC ChE inhibition is less certain because the ChEs in blood have no known physiological function. The lowest NOEL for overt toxicity in the subchronic toxicity

[5] Estimated assuming a 235 g Sprague Dawley rat consumes 22 g of feed per day (U.S. EPA, 1988).

studies was 5 ppm (0.5 mg/kg/day) based on brain ChE inhibition and reduced body weights in rats. The NOEL for plasma and RBC ChE inhibition in this study was also 5 ppm (0.5 mg/kg/day). The NOEL for overt toxicity in the rat inhalation study was 4.72 mg/m^3 (1.26 mg/kg/day), the highest dose tested. The NOEL for plasma and RBC ChE inhibition in the rat inhalation study was 1.24 mg/m^3 (0.32 mg/kg/day). The NOEL for plasma and RBC ChE inhibition in the human oral study was similar at 0.29 mg/kg/day.

VI. CHRONIC TOXICITY

A. Introduction

Seven chronic toxicity studies were available for azinphos-methyl, including two mouse studies, three rat studies and two dog studies. The studies varied in duration from 1-year (dogs) to 104 weeks (rats and mice). Only three studies, one in each species, were acceptable to DPR toxicologists based on the FIFRA guidelines.

B. Oral Studies

1. Mice

Azinphos-methyl (90%) was administered to 50 male B6C3F1 mice/dose at 31.3 or 62.5 ppm (5.4 and 10.8 mg/kg/day)[6] and to 50 female B6C3F1 mice/dose at 62.5 and 125 ppm (10.8 and 21.5 mg/kg/day) for 80 weeks (NCI, 1978). Ten mice/sex were used as controls. The animals were observed for another 12-13 weeks after dosing stopped, then sacrificed. The body weights were reduced in females at 125 ppm. Several treatment-related clinical signs were observed intermittently during the second year of the study including rough hair coat (males at 31.3 and 62.5 ppm), hyperactivity (females at 62.5 and 125 ppm), and convulsions (one male at 62.5 ppm and one female 125 ppm). The only apparent dose-related increase in non-neoplastic lesions was in the incidence of cystic endometrial hyperplasia in females (2/7, 32/48, 32/48 or 29%, 67%, 67%, respectively). The NOEL was less than 31.3 ppm (5.4 mg/kg/day) based on the clinical signs in both sexes and cystic endometrial hyperplasia in females. This study was unacceptable to DPR toxicologists due to major deficiencies (no individual data, inadequate number of concurrent control animals, and too few dose levels).

An oncogenicity study was conducted in which 50 CD1 mice/sex/dose were fed azinphos-methyl (86.7%) in the diet at 0 (corn oil), 5, 20, or 40 ppm (M: 0, 0.79, 3.49 or 11.33 mg/kg/day; F: 0, 0.98, 4.12 or 14.30 mg/kg/day) for 104 weeks (Hayes, 1985). No significant compound-related effects were seen in feed consumption, body weight, organ weight, clinical signs, mortality, hematology, and incidence of gross and histopathological lesions. At the study termination, the mean plasma, ChE activity was reduced in the 5 ppm (M: 91% of controls), 20 ppm (M: 69%; F: 78% of controls) and 40 ppm (M: 44%; F: 33% of controls) animals. A reduction in the mean RBC ChE activity was also seen at 5 ppm (M: 84%: F: 78% of controls), 20 ppm (M: 56%; F: 51% of controls), and 40 ppm (M: 37%; F: 41% of controls). In addition, the mean brain ChE activity was depressed at 5 ppm (M: 88%; F: 94% of controls), 20 ppm (M: 84%; F: 74% of controls) and 40 ppm (M: 37%; F: 33% of controls). The NOEL appears to be less than 5 ppm (M: 0.79 mg/kg/day; F: 0.98 mg/kg/day) based on the plasma, RBC and brain ChE inhibition. DPR toxicologists considered this study acceptable based on FIFRA guidelines.

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[6] Estimated assuming a 36 g B6C3F1 mouse consumes 6.2 g feed per day (U.S. EPA, 1988).

2. Rats

In a study conducted by Lorke (1966a) azinphos-methyl (purity not reported) was administered to 40 Wistar derived rats/sex/dose at 0, 5, 20, or 50 ppm (increased to 100 ppm at 45 weeks) (M: 0, 0.21, 0.78 or 3.01 mg/kg/day; F: 0, 0.26, 1.07 or 4.14 mg/kg/day) in the diet for 97 weeks. A low dose of 2.5 ppm (M: 0.10 mg/kg/day; F: 0.12 mg/kg/day) was started 6 months into the study with its own controls. At 50→100 ppm convulsions were observed in several females 7 weeks after the dose level was increased to 100 ppm. There was no effect on growth, food consumption, food utilization, hematology, urinalysis, macroscopic or microscopic findings at any dose level. At the end of the study, the mean plasma ChE activity was slightly depressed (M: 82%; F: 90% of control activity) in the 20 ppm group. In the 50→100 ppm animals, the mean ChE activity were reduced in the plasma (M: 70%; F: 76% of controls), RBCs (M & F: 67% of controls), and brain (M: 81%; F: 51% of controls). The NOEL for overt toxicity was 20 ppm (M: 0.78 mg/kg/day; F: 1.07 mg/kg/day) based on the convulsions, RBC and brain ChE inhibition. The NOEL for plasma ChE inhibition was 5 ppm (M: 0.21 mg/kg/day; F: 0.26 mg/kg/day). DPR toxicologists found this study unacceptable due to major deficiencies including no analysis of the test article or diet, limited pathology and clinical chemistry, and high mortality rate in all groups (55-85%).

Azinphos-methyl (90%) was administered to 50 Osborne-Mendel rats/dose in the diet at 78 or 156 ppm (5.7 or 11.4 mg/kg/day)[7] to males and at 62.5 or 125 ppm (4.6 or 9.2 mg/kg/day) to females for 80 weeks (NCI, 1978). Ten rats/sex were used as concurrent controls. The animals were observed for another 34-35 weeks after dosing stopped, then sacrificed. Reduced body weights were observed in males at 78 and 156 ppm and in females only at 125 ppm. Tremors were observed in males at 156 ppm and in females at 125 ppm after the first week. At week 34, exophthalmos (which progressed to unilateral or bilateral blindness) was observed in 15 females at 125 ppm. There were no treatment-related increases in non-neoplastic lesions. The apparent NOEL for this study was less than 78 ppm (5.7 mg/kg/day), the lowest dose tested, based on the reduced body weights in males. DPR toxicologists found this study unacceptable due to the lack of individual data, the use of pooled control data, an inadequate exposure period and an inadequate number of treatment groups.

Groups of 60 SPF Wistar rats/sex/group were fed azinphos-methyl (87.2%) in the diet at 0 (vehicle = 1% peanut oil), 5, 15 or 45 ppm (M: 0, 0.25, 0.75 or 2.33 mg/kg/day; F: 0, 0.31, 0.96 or 3.22 mg/kg/day) for 24 months (Schmidt and Chevalier, 1984). Ten rats/sex/group were sacrificed at 12 months. The only compound-related clinical sign was an increased incidence of alopecia at 45 ppm after 4 weeks (M: 8, 4, 5, 15; F: 18, 22, 26, 49). The mean body weights of males at 45 ppm were significantly reduced (up to 10%). Feed consumption was slightly increased in the females at 45 ppm (~10%). There were no treatment-related effects on survival rate, clinical chemistry, hematology, urinalysis, gross pathology, and histopathology. At 24 months, the mean plasma, RBC and brain ChE activities were reduced at 15 and 45 ppm (Table

[7] Estimated assuming a 450 g Osborne-Mendel rat consumes 33 g of feed per day (U.S. EPA, 1988).

7). The NOEL was 5 ppm (M: 0.25 mg/kg/day; F: 0.31 mg/kg/day) based on the plasma and RBC ChE inhibition in both sexes and the brain ChE inhibition in females. This study was acceptable to DPR toxicologists.

3. Dogs

Four cocker spaniel dogs/sex/dose were fed azinphos-methyl (purity not reported) in the diet at 0, 5, 20, 50 ppm for two years (Lorke, 1966b). The high dose level was raised from 50 to 300 ppm in 4 steps (M: 4.25 mg/kg/day; F: 4.62 mg/kg/day). The intermediate dose level was raised from 20 to 50 ppm in 2 steps (M & F: 1.27 mg/kg/day) and the lowest dose level was kept at 5 ppm (M: 0.17 mg/kg/day; F: 0.19 mg/kg/day). Within one week after increasing the high dose level to 300 ppm, the dogs in this group exhibited tremors, muscular weakness, inactivity, and abnormal sitting posture. One male died during week 94 after receiving 300 ppm in the diet for 9 weeks. This dog had ataxia, lacrimation, increased respiratory rate, labored breathing, miosis, vomiting, and jaundice the week before it died. The necropsy of this dog revealed that the gallbladder and common bile duct were grossly distended, but not obstructed. The liver was congested, but otherwise normal in appearance. Although the death of this dog was attributed to cholangitis, investigators did not consider the cholangitis treatment-related since the only other hepatic abnormalities in the other dogs were an occasional focus of cellular infiltration. There was a slight reduction in the mean body weights (~5-15%) at 300 ppm and in the mean food consumption (6-10%) at 150→300 ppm. The mean plasma and RBC ChE activities were reduced at 20→50 ppm (84% and 71% of controls, respectively) and 50→300 ppm (52% and 17% of controls, respectively). Brain ChE activity was not measured. There were no treatment-related changes in the hematology, clinical chemistry, urinalysis, macroscopic or microscopic lesions. The apparent NOEL for overt toxicity was 20→50 ppm (M & F: 1.27 mg/kg/day) based on the death, clinical signs, and reduced body weight and food consumption. The NOEL for plasma and RBC ChE inhibition was 5 ppm (M: 0.17 mg/kg/day; F: 19 mg/kg/day). DPR toxicologists found this study unacceptable due to major deficiencies including incomplete reporting of data, no analysis of test article and diet, and frequent dose level changes.

In another chronic study, 4 beagle dogs/sex/group were fed azinphos-methyl (91.9%) in the diet at 0, 5, 25 or 125 ppm (M: 0, 0.15, 0.69 or 3.84 mg/kg/day; F: 0, 0.16, 0.78 or 4.33 mg/kg/day) for 52 weeks (Allen, 1990). There was no dose-related difference in the number of dogs exhibiting clinical signs during the study. Although the number of dogs with diarrhea and mucus in feces did not exhibit a clear dose-relationship, the frequency of these signs appeared to be dose-related (Table 8). The frequency of diarrhea increased noticeably after the first month, especially in the females at 125 ppm, and remained fairly constant through the remainder of the study with some periodic decreases. The frequency of diarrhea in males at 25 ppm and in both sexes at 125 ppm was highly significant by pair-wise comparison with controls; however, the trend in males was only slightly significant because the frequency decreased from 25 to 125 ppm. Some occurrences of diarrhea in this study do not appear to be treatment-related because some dogs had diarrhea during the pretreatment period. The male dog at 25 ppm with the highest frequency of diarrhea (41 of 71 occurrences) during treatment also had diarrhea during the pretreatment period. Even if this animal is ignored, the frequency at this dose level (30 occurrences) is still higher than the occurrences in the control group (8 occurrences). The

Table 7. Cholinesterase Activity in Plasma, Red Blood Cells and Brain of Rats Fed Azinphos-methyl in the Diet for 24 Months[a]

Tissue	Dose Level (ppm)		
	5	15	45
	MALES		
Month 6			
Plasma	88%[b]*	95%	57%**
RBC[c]	97%	90%	80%**
Month 12			
Plasma	84%*	87%	54%**
RBC	102%	82%*	73%**
Brain	130%**	137%**	109%
Month 18			
Plasma	87%	90%	55%**
RBC	96%	83%**	73%**
Month 24			
Plasma	113%	88%	51%**
RBC	88%**	78%**	63%**
Brain	117%	112%	68%**
	FEMALES		
Month 6			
Plasma	92%	71%*	34%**
RBC	109%*	86%**	77%**
Month 12			
Plasma	90%	65%**	33%**
RBC	101%	81%**	69%**
Brain	112%	90%	50%**
Month 18			
Plasma	100%	74%*	46%**
RBC	94%*	78%**	63%**
Month 24			
Plasma	102%	81%	38%**
RBC	98%	84%**	71%**
Brain	102%	79%**	45%**

a Schmidt and Chevalier, 1984.

b Percent of control activity. Ten animals per sex per dose level tested.

c RBC = red blood cell

*,** Significantly different from controls by the Mann-Whitney U-test and the Wilcoxon rank sum test at $p < 0.05$ and 0.01, respectively

Table 8. Frequency of Diarrhea and Mucus in the Feces in Dogs Fed Azinphos-Methyl for 52 Weeks[a]

	Dose Level (ppm)[b]			
	0	5	25	125
MALES				
Diarrhea	8[c+]	5	71***	30***
	(4/4)[d]	(3/4)	(4/4)	(3/4)
Mucus in Feces	1[+++]	0	22***	32***
	(1/4)	(0/4)	(4/4)	(3/4)
FEMALES				
Diarrhea	58[+++]	40	44	275***
	(3/4)	(4/4)	(4/4)	(4/4)
Mucus in Feces	75[+]	9	18	58
	(4/4)	(4/4)	(2/4)	(4/4)

a Allen, 1990

b Actual test compound intake at 5, 25 and 125 ppm was 0.15, 0.69 or 3.84 mg/kg/day, respectively, in males and 0.16, 0.78 or 4.33 mg/kg/day, respectively, in females.

c Total number occurrences of this sign during a total possible 1460 observations (4 dogs x 365 days).

d Number of dogs exhibiting this sign at any time during the study.

+,+++ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively.

*** Significantly difference from the control group based on the Fisher's exact test at $p < 0.001$.

interpretation of the increase in frequency of diarrhea in males is also confounded by the fact the frequency of diarrhea in males at 25 and 125 ppm was similar to the frequency of diarrhea in control females. It is possible there is a gender-related difference in the normal frequency of diarrhea or it could be the control and low dose males had an unusually low frequency. Closer examination of the frequency of diarrhea in control females revealed that most occurred in one female (43 of 58 occurrences). This control female also had diarrhea during the week before treatment began. If this control female is eliminated, the frequency in the female controls (15 occurrences) is more similar to male controls (8 occurrences). On the other hand, if this control female is ignored, the frequency of diarrhea in females at 5 and 25 ppm now appears to be elevated. The apparent increase in frequency in diarrhea in these two groups could not be attributed to any one dog and no female dogs in these groups had diarrhea during the pretreatment period. Furthermore, no plasma or RBC ChE inhibition was observed at the lowest dose level. This would suggest that many of the occurrences of diarrhea at these lower dose levels are unrelated to ChE inhibition. The increase in frequency of diarrhea in females at 125 ppm seems more likely to be treatment-related. Then again, one female dog had the vast majority of occurrences of diarrhea (190 of 275 occurrences) at 125 ppm. This dog did not have diarrhea during pretreatment, but it did have mucus in the feces. Elimination of this dog would decrease the frequency in this group to 85 occurrences, which still appears to be higher than the approximately 40 occurrences per group in the two lower dose groups. Since diarrhea is a known cholinergic sign and it was not possible to state with absolute certainty that all occurrences of diarrhea were unrelated to treatment, a health protective assumption was made that the diarrhea was cholinergic in origin and, thus, treatment-related. The toxicological significance of the

diarrhea is supported by a range-finding study where more overt cholinergic signs (muscle spasms and tremors) were seen in dogs fed azinphos-methyl at 100 ppm for 19 weeks (Löser and Lorke, 1967).

At week 52, the mean ChE activity were significantly reduced in the plasma (M & F: 47% of controls), RBCs (M & F: 14% of controls), and brain (M: 73%; F: 80% of control activity) at 125 ppm. The mean RBC ChE activity was also lower (M: 73%; F: 65% of controls) at 25 ppm, although the reduction was only statistically significant for females. The mean activity of liver cytochrome P-450 was significantly higher (39%) at 125 ppm in the males. The mean activities of N-demethylase were also higher (30-34%) in both sexes at 125 ppm, but the differences were not statistically significant. Males at 125 ppm had slightly lower mean plasma albumin levels (7-13%). The mean liver and spleen weights were lower in males at all dose levels (14-21% and 30-65%, respectively). The mean kidney weights were lower in males at 125 ppm (17%). The toxicological significance of the changes in enzyme activities and organ weights is uncertain given there were no accompanying histological changes. Furthermore, the liver and kidney weights were not significantly different from the controls when compared relative to their body weights. There was no compound-related effect on mortality, body weight, food consumption, hearing, ophthalmology, hematology, urinalysis, macroscopic or microscopic observations. The NOEL was 5 ppm (M: 0.15 mg/kg/day; F: 16) based on the RBC ChE inhibition and diarrhea. This study was considered an acceptable by DPR toxicologists.

C. Conclusions

The non-oncogenic effects observed with chronic exposure to azinphos-methyl included cholinergic signs, blindness, jaundice, reduced body weights, plasma, RBC and brain ChE inhibition, cystic endometrial hyperplasia and cholangitis. The most sensitive endpoints were diarrhea and ChE inhibition. The lowest established chronic NOEL was 5 ppm (M: 0.15 mg/kg/day; F: 0.16 mg/kg/day) based on diarrhea and RBC ChE inhibition in dogs fed azinphos-methyl for 1 year.

VII. ONCOGENICITY

A. Introduction

Five of the 7 chronic toxicity studies for azinphos-methyl evaluated animals for potential oncogenic effects. Two of these studies were conducted with mice and three with rats. Only one mouse and one rat study were found acceptable to DPR toxicologists.

B. Oral Studies

1. Mice

Azinphos-methyl (90%) was administered to 50 male B6C3F1 mice/dose at 31.3 or 62.5 ppm (5.4 and 10.8 mg/kg/day)[8] and to 50 female B6C3F1 mice/dose at 62.5 and 125 ppm (10.8 and 21.5 mg/kg/day) for 80 weeks (NCI, 1978). Ten mice/sex were used as controls. Because there were so few animals in the concurrent control group, the investigators "pooled" control mice of the same strain from several other bioassays from this laboratory to perform their statistical analysis of the tumor incidence (i.e., the "pooled" controls are the concurrent controls plus control animals from 11 other studies conducted by this laboratory that were started no more than 3 months earlier or later than the azinphos-methyl study). The animals were observed for another 12-13 weeks after dosing stopped, then sacrificed. There was an increase in the combined incidence of hepatocellular adenomas and carcinomas in male mice at 62.5 ppm (Table 9). Only the combined increase was significant by Fisher's exact test when compared with

Table 9. Incidence of Neoplastic Lesions in the Liver of Male Mice Fed Azinphos-Methyl for 80 Weeks[a]

	Dose Level (ppm)[b]			
Lesion	Pooled Controls	Concurrent Controls	31.3	62.5
Hepatocellular adenoma	NR	2/8 (25%)	8/49 (16%)	7/50 (14%)
Hepatocellular carcinoma	27/128 (21%)	0/8 (0%)	3/49 (6%)	12/50 (24%)
Combined	30/128[+] (23%)	2/8 (25%)	11/49 (22%)	19/50* (38%)

a The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.
b The test compound intake was estimated to be 5.4 and 10.8 mg/kg/day for 31.3 and 62.5 ppm, respectively, assuming a 36 g $B6C3F_1$ mouse consumes 6.2 g feed per day (U.S. EPA, 1988).
NR Not reported
[+] Significant trend based on the Cochran-Armitage trend test at $p < 0.05$ (Gart *et al.*, 1986).
* Significantly different from the pooled control group based on the Fisher's exact test at $p < 0.05$.

[8] Estimated assuming a 36 g $B6C3F_1$ mouse consumes 6.2 g feed per day (U.S. EPA, 1988).

pooled controls. It also exhibited a significant trend by the Cochran-Armitage trend test. The investigators did not consider this increase treatment-related because similar high incidences of this tumor had been observed in male mice in this same laboratory; however, no historical control range or mean were reported for these tumors. This study was unacceptable to DPR toxicologists due to major deficiencies (inadequate number of concurrent control animals, too few dose levels and no individual data,).

An oncogenicity study was conducted in which 50 CD1 mice/sex/dose were fed azinphos-methyl (86.7%) in the diet at 0 (corn oil), 5, 20, or 40 ppm (M: 0, 0.79, 3.49 or 11.33 mg/kg/day; F: 0, 0.98, 4.12 or 14.30 mg/kg/day) for 104 weeks (Hayes, 1985). No significant compound-related effects were seen in feed consumption, body weight, organ weight, clinical signs, mortality, hematology, and incidence of gross and histopathological lesions. However, plasma, RBC and brain ChE activities were significantly reduced at all dose levels (see Chronic Toxicity section for more details). DPR toxicologists considered this study acceptable based on FIFRA guidelines.

2. Rats

In a study conducted by Lorke (1966a) azinphos-methyl (purity not reported) was administered to 40 Wistar derived rats/sex/dose at 0, 5, 20, or 50 ppm (increased to 100 ppm at 45 weeks) (M: 0, 0.21, 0.78 or 3.01 mg/kg/day; F: 0, 0.26, 1.07 or 4.14 mg/kg/day) in the diet for 97 weeks. A low dose of 2.5 ppm (M: 0.10 mg/kg/day; F: 0.12 mg/kg/day) was started 6 months into the study with its own controls. At 50→100 ppm convulsions were observed in several females 7 weeks after the dose level was increased to 100 ppm. There was no effect on growth, food consumption, food utilization, hematology, urinalysis, macroscopic or microscopic findings at any dose level. DPR toxicologists found this study unacceptable due to major deficiencies including no analysis of the test article or diet, limited pathology and clinical chemistry, and high mortality rate in all groups (55-85%).

Azinphos-methyl (90%) was administered to 50 Osborne-Mendel rats/sex in the diet at 78 or 156 ppm (5.7 or 11.4 mg/kg/day)[9] to males and at 62.5 or 125 ppm (4.6 or 9.2 mg/kg/day) to females for 80 weeks (NCI, 1978). Ten rats/sex were used as concurrent controls. The animals were observed for another 34-35 weeks after dosing stopped, then sacrificed. The incidence of tumors in the pituitary gland (chromophobe adenoma or carcinoma), pancreas (islet cell adenoma or adenocarcinoma), thyroid gland (adenoma, adenocarcinoma, follicular cell adenoma, cystadenoma, cystadenocarcinoma, papillary cystadenocarcinoma), parathyroid gland (adenomas) and adrenal glands (cortical adenoma or adenocarcinoma) in males was increased at 78 and/or 156 ppm (Table 10). The "pooled" controls are the concurrent controls plus control rats of the same strain from 10 other studies conducted by this laboratory that were started no more than 3 months earlier or later than the azinphos-methyl study. When compared to concurrent controls, the incidence was not statistically significant for any of these tumors by the Fisher's exact test.

[9] Estimated assuming a 450 g Osborne-Mendel rat consumes 33 g of feed per day (U.S. EPA, 1988).

Table 10. Incidence of Neoplastic Lesions in Male Rats Fed Azinphos-Methyl for 80 Weeks[a]

	Dose Level (ppm)[b]			
	Pooled Controls	Concurrent Controls	78	156
Pituitary				
Chromophobe adenoma	13/85^{+} (15%)	4/9 (44%)	21/46** (46%)	13/43** (30%)
Combined - chromophobe adenoma or carcinoma	13/85^{++} (15%)	4/9 (44%)	21/46** (46%)	15/43** (35%)
Pancreas				
Islet-cell adenoma	2/92^{+} (2%)	0/9 (0%)	1/47 (2%)	4/45 (9%)
Islet-cell carcinoma	NR	0/9 (0%)	0/47 (0%)	2/45 (4%)
Combined - islet cell adenoma or carcinoma	2/92^{++} (2%)	0/9^{+} (0%)	1/47 (2%)	6/45* (13%)
Thyroid				
Cystadenoma	NR	0/9^{+} (0%)	7/44 (16%)	10/43 (23%)
Combined - cystadenoma, follicular-cell adenoma or adenoma	7/86^{++} (8%)	1/9 (11%)	10/44* (23%)	12/43** (28%)
Adenocarcinoma	NR	0/9 (0%)	3/44 (7%)	3/43 (7%)
Combined - adenocarcinoma, cystadenocarcinoma or papillary cystadenocarcinoma	0/86^{++} (0%)	0/9 (0%)	4/44* (9%)	4/43* (9%)
Combined - all follicular-cell tumors	7/86^{+++} (8%)	1/9 (11%)	14/44*** (32%)	14/43*** (33%)
Parathyroid				
Adenoma	1/81^{++} (1%)	1/5 (20%)	0/26 (0%)	4/24** (17%)
Adrenal Gland				
Adenocarcinoma	0/95^{++} (0%)	0/9 (0%)	1/45 (2%)	3/46* (7%)
Cortical adenoma	NR	1/9 (11%)	3/45 (7%)	7/46 (15%)
Combined – adenocarcinoma or cortical adenoma	3/95^{+++} (3%)	1/9 (11%)	4/45 (9%)	10/46*** (22%)

a The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.
b The test compound intake was estimated to be 5.7 and 11.4 mg/kg/day for 78 and 156 ppm, respectively, assuming a 450 g Osborne-Mendel rat consumes 33 g of feed per day (U.S. EPA, 1988).
NR Not reported
$^{+}$, $^{++}$, $^{+++}$ Significant trend based on the Cochran-Armitage trend test at $p < 0.05$, 0.01 and 0.001, respectively (Gart *et al.*, 1986).
*, **, *** Significantly different from the pooled control group based on the Fisher's exact test at $p < 0.05$, 0.01, 0.001, respectively.

However, when compared to "pooled" controls, the incidence of these tumors was significantly higher. Using concurrent controls, significant trends were found only with the combined incidence of pancreatic islet-cell tumors and with the incidence of thyroid cystadenoma. With pooled controls, highly significant trends were found in the incidences of tumors in the pituitary, pancreas, thyroid, parathyroid and adrenal gland. The toxicological significance of the increase in pituitary and parathyroid tumors is uncertain because the incidence in the concurrent controls was higher than pooled controls. Comparison with pooled controls is problematic in that the same pathologist did not examine the azinphos-methyl study animals and the pooled controls. The incidence of the combined pancreatic islet cell adenomas and carcinomas was within the reported historical control range for male Osborne-Mendel rats at this laboratory (0 to 22% with a mean of 2%). The incidence of thyroid follicular-cell tumors was also within the reported historical control range for this laboratory (0 and 43% with a mean of 7%). Therefore, the investigators concluded that the increase in pancreatic and thyroid tumors was not clearly treatment-related. DPR toxicologists found this study unacceptable based on FIFRA guidelines due to the use of pooled control data, inadequate exposure duration, inadequate number of treatment groups and lack of individual data.

Groups of 60 SPF Wistar rats/sex/group were fed azinphos-methyl (87.2%) in the diet at 0 (vehicle = 1% peanut oil), 5, 15 or 45 ppm (M: 0, 0.25, 0.75 or 2.33 mg/kg/day; F: 0, 0.31, 0.96 or 3.22 mg/kg/day) for 104 weeks (Schmidt and Chevalier, 1984). Ten rats/sex/group were sacrificed at 12 months. Alopecia, body weight reductions and ChE inhibition were observed at 45 ppm (see discussion of this study in the Chronic Toxicity section). There were no treatment-related effects on survival rate, clinical chemistry, hematology, urinalysis, gross pathology, and histopathology at 12 or 24 months (104 weeks). This study was acceptable to DPR toxicologists based on FIFRA guidelines.

Conclusions

An increase in tumors was seen in two of the five oncogenicity studies. In a mouse study conducted by NCI, an increase in the combined incidence of hepatocellular adenomas and carcinomas in male mice was seen at the highest dose tested. Although statistically significant when compared with pooled controls, the increase was reported to be similar to high incidences observed in other male mice control groups for this same laboratory. This study had major deficiencies, the most significant being an inadequate number of concurrent control animals. There was no similar increase in liver tumors in another oncogenicity study in mice that was found acceptable to DPR toxicologists based on FIFRA guidelines. Increases in various endocrine tumors (pituitary, pancreatic, thyroid, parathyroid and adrenal gland) were seen in male rats in another study conducted by NCI. These increases were also only significant when compared to pooled controls, not concurrent controls. As with the NCI mouse study, the inadequate number of concurrent controls in the NCI rat study made interpretation of the findings difficult. There was no significant increase in endocrine tumors in two other rat oncogenicity studies, one of which was found acceptable to DPR toxicologists based on the FIFRA guidelines. The higher dose levels and different strain of rats used in the NCI study may have been contributing factors to the increase in endocrine tumors in this study.

VIII. GENOTOXICITY

A. Introduction

A number of genotoxicity studies were available for azinphos-methyl. There were twelve gene mutation studies for azinphos-methyl: one *Drosophila* sex-linked recessive lethal assay, seven *in vitro* reverse-mutation assays, and four *in vitro* forward mutation assays. Two of the reverse mutation assays were found acceptable to DPR toxicologists based on the FIFRA guidelines. Seventeen tests for structural chromosomal aberrations were conducted with azinphos-methyl. Eight of these tests were *in vivo* and nine were *in vitro*. Eleven other genotoxicity tests were conducted on azinphos-methyl including two unscheduled DNA synthesis assays, two differential toxicity tests, six mitotic recombination/gene conversion and/or crossing over tests, and one ^{32}P-postlabeling assay of DNA adducts.

B. Gene Mutation

The results from only one *in vivo* gene mutation assay for azinphos-methyl was available for evaluation (Table 11). This study, a sex-linked recessive lethal assay with *Drosophila melanogaster*, was conducted for the U.S. EPA under contract (Valencia, 1981). There was no evidence of a mutagenic effect based on the percentage of cultures in the F_2 generation without wild-type males.

Numerous *in vitro* gene mutation assays have been conducted for azinphos-methyl including both forward and reverse mutation assays (Table 11). No significant increase in the mutation frequency was observed in a reverse mutation assay (Ames assay) in which *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to azinphos-methyl (92.3%) at concentrations up to 2,500 µg/plate (Herbold, 1978). This assay was unacceptable to DPR toxicologists due to several deficiencies, including no individual data, no positive controls that did not require metabolic activation, and no justification of dose levels. Similar results were obtained when this same investigator repeated this assay with the same strains exposed to azinphos-methyl (92.5%) up to 9,600 µg/plate with and without metabolic activation (Herbold, 1988). This assay was considered acceptable by DPR toxicologists. In another acceptable Ames assay, azinphos-methyl (88.8%) was tested at concentrations up to 4,000 µg/plate using TA98, TA100, TA1535, TA1537, and TA1538 strains with and without metabolic activation (Lawlor, 1987). No mutagenic response was clearly identified, although an equivocal response was observed for TA100. This study was acceptable to DPR toxicologists. The results were also negative in three published reports of Ames assays for azinphos-methyl (Simmon *et al.*, 1976: TA100, TA1535, TA1537, TA1538; Garrett *et al.*, 1986: TA1537, TA98, TA100; Carere *et al.*, 1978: TA1535, TA1536, TA1537, TA1538). There was one published report of a weak mutagenic response using TA98 with activation (Zeiger *et al.*, 1987). However, the increase in mutation frequency was only observed at 3,333 µg/plate and above where precipitation occurred, confounding the results. A registrant also submitted a reverse mutation assay using *Saccharomyces cerevisiae* strains S128 and S211a (Hoorn, 1983). The results from this assay were negative, but this study was unacceptable to DPR toxicologists based on an inadequate description of methods and materials.

Table 11. The Effects of Azinphos-methyl on Gene Mutation

Test Type/System	Strain	Dose	S9	Results	Comments/Reference
In Vivo					
Sex-linked recessive lethal	*Drosophila melanogaster*	0, 0.25, 0.5, 1.0 ppm	NA	Neg.	U.S. EPA document (Valencia, 1981)
In Vitro - Reverse Mutation					
S. typhimurium	TA98, TA100, TA1535, TA1537	0, 75, 150, 300, 600, 1200, 2400, 4800, 9600 µg/plate	±	Neg.	Acceptable (Herbold, 1988)
S. typhimurium	TA98, TA100, TA1535, TA1537, TA1538	0, 33, 100, 333, 1000, 2000, 4000 µg/plate	±	Neg.	Acceptable; Equivocal effect with TA100±S9 (Lawlor, 1987)
S. typhimurium	TA100, TA1535, TA1537, TA1538	**Not Reported**	±	Neg.	Published article (Simmon *et al.*, 1976)
S. typhimurium	TA98, TA100, TA1537	Up to 1000 µg/plate	±	Neg.	Published article (Garrett *et al.*, 1986)
S. typhimurium	TA1535, TA1536, TA1537, TA1538	**Not reported**	**NR**	Neg.	Published article (Carere *et al.*, 1978)
S. typhimurium	TA98, TA100, TA1535, TA1537	0, 100, 333, 1000, 3333, 10000 µg/plate	±	Pos.	Published article; weakly positive with TA98+S9 (Zeiger *et al.*, 1987)
Saccharomyces cerevisiae	S128, S211a	0, 33, 100, 333, 1000, 3333, 10000 µg/plate	±	Neg.	Unacceptable (Hoorn, 1983)
In Vitro - Forward Mutation					
Mouse lymphoma	L5178Y Tk+/-	Up to 1,000 µg/ml	–	Pos.	Published article (Garrett *et al.*, 1986)
Streptomyces Coelicolor	A3(2), hisAI	**Not reported**	**NR**	Neg.	Published article (Carere *et al.*, 1978)
Schizosaccharomyces pombe	ade6	**Not reported**	±	Neg.	Published abstract (Degraeve *et al.*, 1980)
S. pombe	ade6	3-95 mM	±	Pos.	Published article; positive response without S9 only (Gilot-Delhalle *et al.*, 1983)

S9 = Supernatant from rat liver homogenates centrifuged at 9,000 x g which contain enzymes for metabolic activation.
NA = Not applicable
NR = Not reported

There are also several published reports of forward mutation assays for azinphos-methyl. The results from the L5178Y TK+/- mouse lymphoma forward mutation assay were positive without metabolic activation (Garrett *et al.*, 1986). Azinphos-methyl was not tested in this system with metabolic activation. A forward mutation assay with *Streptomyces coelicolor* was negative (Carere *et al.*, 1978). The findings in two reports from the same laboratory using a forward mutation assay with *Schizosaccharomyces pombe* ade6 were inconsistent. Degraeve and coworkers (1980) reported negative results; however, Gilot-Delhalle and coworkers (1983) reported positive results without metabolic activation. The differences in the findings are difficult to interpret since few details were given in the earlier report. Both appear to have tested azinphos-methyl with and without metabolic activation. The concentrations tested were not reported in the earlier study.

C. Structural Chromosome Aberrations

All the *in vivo* tests for structural chromosome aberrations were negative (Table 12a). In one of two dominant lethal assays submitted by registrants, 12 male albino mice/dose were administered azinphos-methyl (purity not reported) intraperitoneally at 0, 125 or 250 µg/kg (Arnold, 1971). This study was considered invalid by the registrant and unacceptable to DPR toxicologists due to insufficient information. In the second dominant lethal assay, 50 male NMRI mice were administered azinphos-methyl (92.3%) by oral gavage at 0 and 4 mg/kg (Herbold, 1979a). DPR toxicologists also found this study unacceptable due to insufficient information, only one dose level tested, and no positive control tested. Published reports of two dominant lethal assays for azinphos-methyl in mice were also negative (Degraeve *et al.*, 1986; Garrett *et al.*, 1986). In a micronucleus assay, 5 NMRI mice/sex/dose were administered azinphos-methyl (92.3%) by gavage at 0, 1.25, 2.5 or 5 mg/kg in 2 doses 24 hrs apart and sacrificed 6 hours later (Herbold, 1979b). This study was unacceptable to DPR toxicologists due to major deficiencies (no pilot study data, no clinical observations or pathology on the animal that died, no signs of toxicity at the high dose). A published report of a micronucleus assay in mouse bone marrow was also negative (Garrett *et al.*, 1986). In addition, two other published *in vivo* tests for structural chromosome aberrations were negative, including a cytogenetics test using mice (Q strain) spermatocytes and bone marrow cells (Degraeve *et al.*, 1986) and a sister chromatid exchange assay using central mudminnows, *Umbra limi* (Vigfusson *et al.*, 1983).

There are several reports of positive results for structural chromosome aberrations *in vitro* (Table 12b). In a study submitted by a registrant, an increase in chromosome aberrations (except gaps) was observed in human lymphocytes exposed to azinphos-methyl (91.9%) at 500 µg/ml with activation (Herbold, 1986). There was no increase in aberrations at any concentration without activation. This study was acceptable to DPR toxicologists. There are three published reports of cytogenetic tests which were also positive. In one study conducted by Alam and coworkers (1974), Chinese hamster cells (CHO-Kl) were exposed to azinphos-methyl (90%) at concentrations of 60 to 120 µg/ml. In another study from the same laboratory, two human cell lines (WI-38 and HEp-2) were exposed to azinphos-methyl (90%) at 120 to 160 µg/ml (Alam and Kasatiya, 1976). Trépanier and coworkers (1977) exposed cells from a human lymphoblastoid cell line (L-MOORE) at 60 µg/ml. In all three studies, the most common chromosome

Table 12a. The Effects of Azinphos-methyl on Chromosomal Aberrations - In Vivo Assays

Test Type/System	Strain	Dose	S9	Results	Comments/Reference
Dominant lethal	Albino mice	0, 125, 250 µg/kg	NA	Neg.	Unacceptable (Arnold, 1971)
Dominant lethal	NMRI mice	0, 4 mg/kg	NA	Neg.	Unacceptable (Herbold, 1979a)
Dominant lethal	Q strain mice	1 mg/kg	NA	Neg.	Published article (Degraeve *et al.*, 1986)
Dominant lethal	Mice, strain not reported	Up to 100 mg/kg	NA	Neg.	Published article (Garrett *et al.*, 1986)
Micronucleus	NMRI mice, bone marrow	0, 1.25, 2.5, 5 mg/kg	NA	Neg.	Unacceptable (Herbold, 1979b)
Micronucleus	Mice, bone marrow	Up to 10 mg/kg	NA	Neg.	Published article (Garrett *et al.*, 1986)
Cytogenetic	Q strain mice, spermatocytes and bone marrow	1 mg/kg	NA	Neg.	Published article (Degraeve *et al.*, 1986)
Sister chromatid exchange	Central mudminnows, *Umbra limi*	0, 0.54 & 5.4 x 10^{-10} M	NA	Neg.	Published article (Vigfusson *et al.*, 1983)

S9 = Supernatant from rat liver homogenates centrifuged at 9,000 x g which contain enzymes for metabolic activation.
NA = Not applicable

Table 12b. The Effects of Azinphos-methyl on Chromosomal Aberrations - In Vitro Assays

Test Type/System	Strain	Dose	S9	Results	Comments/Reference
Cytogenetic	Human lymphocytes	500 µg/ml	±	Pos.	Acceptable; positive with S9 only (Herbold, 1986)
Cytogenetic	CHO-K1 cell line	60, 80, 100, 120 µg/ml	**NR**	Pos.	Published article (Alam *et al.*, 1974)
Cytogenetic	Human WI-38 & HEp-2 cell lines	120, 140, 160 µg/ml	**NR**	Pos.	Published article (Alam & Kasatiya, 1976)
Cytogenetic	Human lymphoblastoid cell line (L-MOORE)	60 µg/ml	**NR**	Pos.	Published abstract (Trépanier *et al.*, 1977)
Micronucleus	Human lymphocytes	0.06, 0.6, 6.0 µg/ml	–	Pos.	Published article (Bianchi-Santamaria *et al.*, 1997)
Sister chromatid exchange	Chinese hamster ovary cells	Up to 100 µg/ml	±	Neg.	Published article (Garrett *et al.*, 1986)
Sister chromatid exchange	Chinese hamster V79 cell line	0, 2.5, 5, 10, 20 µg/ml	–	Neg.	Published article (Chen *et al.*, 1982a)
Sister chromatid exchange	Chinese hamster V79 cell line	0, 5, 10, 20, 25 µg/ml	+	Neg.	Published article (Chen *et al.*, 1982b)
Sister chromatid exchange	Chinese hamster V79 cell line	Up to 60 µM	**NR**	Neg.	Published article (Nicholas & Van Den Berghe, 1982)

S9 = Supernatant from rat liver homogenates centrifuged at 9,000 x g which contain enzymes for metabolic activation.
NR = Not reported

aberrations were chromatid breaks and exchanges. Azinphos-methyl induced a statistically significant increase in micronucleus frequency in human lymphocytes *in vitro* without metabolic activation (not tested with activation) at all dose levels tested, but the increase was not dose-related (Bianchi-Santamaria, 1997). The lowest concentration tested was reported to approximate the concentrations found in food. The four published reports of *in vitro* sister chromatid exchange assays were all negative including one using Chinese hamster ovary cells (Garrett *et al.*, 1986) and three using in Chinese hamster V79 cells (Chen *et al.*, 1982a&b; Nicholas and Van Den Berghe, 1982).

Degraeve and coworkers (1985) investigated the synergism of chromosomal damage by azinphos-methyl when given in combination with trichlorfon. Twenty-five male mice (Q strain) were given two consecutive intraperitoneal injections of trichlorfon at 50 mg/kg and azinphos-methyl at 0.5 mg/kg. No increase in chromosomal damage was observed in bone marrow cells, spermatogonia or primary spermatocytes. The frequency of post-implantation losses was also not increased in a dominant lethal assay using 5 of the 25 treated male mice; however, there was an increase in pre-implantation losses during the fourth week of mating which the investigators attributed to the toxic effects of the compounds on the male germ cells.

Several studies evaluated the formation of sister chromatid exchanges in agricultural workers exposed to azinphos-methyl among other pesticides (De Ferrari et al., 1991; Gómez-Arroyo et al., 1992, Lander and Rønne, 1995). Increases in sister chromatid exchanges were reported in two of these studies; however, since exposure was not limited to azinphos-methyl, its unclear what, if any, contribution azinphos-methyl may have had to this increase.

D. Other Genotoxic Effects

Numerous tests for other genotoxic effects were also conducted for azinphos-methyl (Table 13). In a study submitted by a registrant, primary rat hepatocytes did not show an increase in the unscheduled DNA synthesis (UDS) when incubated with technical azinphos-methyl (91.1%) at up to 10.1 µg/ml (Myhr and Brusick, 1983). DPR toxicologists found this study acceptable. Garret and coworkers (1986) also reported negative results from a UDS assay with human lung fibroblasts (WI-38).

There was no evidence of DNA damage in two differential toxicity tests. In a study submitted by the registrant, two *E. coli* pol strains, (K12)p 3478 (repair deficient) and W 3110 were exposed to azinphos-methyl (91.1%) at concentrations up to 10,000 µg/plate (Herbold, 1984). However, this study was unacceptable to DPR toxicologists due to several deficiencies (no individual plate counts, inadequate description of protocol). In a published report by Garret and coworkers (1986), a differential toxicity test with *S. typhimurium* uvrB, rec was also negative.

The results for mitotic recombination, gene crossing-over, and gene non-disjunction from various published reports were inconsistent. Garrett and coworkers (1986) reported positive results for azinphos-methyl using the mitotic recombination assay with *S. cerevisiae* D3 at 10 mg/ml or higher. However, Riccio and coworkers (1981) reported negative results for mitotic

Table 13. Other Genotoxic Effects of Azinphos-methyl

Test Type/System	Strain	Dose	S9	Results	Comments/Reference
Unscheduled DNA synthesis (UDS)	Rat hepatocytes	0, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100 µg/ml	NA	Neg.	Acceptable (Myhr and Brusick, 1983)
UDS	Human lung fibroblasts WI-38	Up to 100 µg/ml	±	Neg.	Published article (Garrett *et al.*, 1986)
Differential toxicity (Pol A test)	*E. coli* W 3110 & (K12)p3478	0, 625, 1250, 2500, 5000, 10000 µg/plate		Neg.	Unacceptable (Herbold, 1984)
Differential toxicity	*S. typhimurium* uvrB, rec	Up to 1000 µg/ml	–	Neg.	Published article (Garrett *et al.*, 1986)
Mitotic recombination	*S. cerevisiae* D3	Up to 10 µg/ml	–	Pos.	Published article (Garrett *et al.*, 1986)
Gene conversion and crossing-over	*S. cerevisiae* D7	Up to 10,000 µg/ml	±	Neg.	Published article (Garrett *et al.*, 1986)
Mitotic recombination, gene conversion, crossing-over, and reverse mutation	*S. cerevisiae* D3 & D7	**Not reported**	±	Neg.	Published abstract (Riccio *et al.*, 1981)
Gene conversion and reverse mutation	*S. cerevisiae* D7	0, 500, 1000, 5000, 10000, 25000 µg/ml	±	Pos.	Published article, weakly positive without S9 (Bianchi *et al.*, 1994)
Gene conversion, crossing-over, and non-disjunction	*Aspergillus nidulans* D7	0, 30, 60 mM	±	Pos.	Published article; positive for crossing-over and non-disjunction at 30 mM only (Vallini *et al.*, 1983)
Point mutations, crossing-over, and non-disjunction	*A. nidulans*	**Not reported**	**NR**	Neg.	Published article (Morpurgo *et al.*, 1977)
^{32}P-Postlabeling of DNA adducts	Calf thymus	1 mM	+	Pos.	Published article (Shah *et al.*, 1997)

S9 = Supernatant from rat liver homogenates centrifuged at 9,000 x g which contain enzymes for metabolic activation.
NA = Not applicable
NR = Not reported

recombination with *S. cerevisiae* D3. They also reported negative results for gene conversion, crossing-over, and reverse mutation with *S. cerevisiae* D7. Bianchi *et al.* (1994) reported significant increases in gene conversions and reverse mutations in *S. cerevisiae* D7 without S9, but not with S9. There was no agreement in similar assays using *Aspergillus nidulans* D7. Morpurgo and coworkers (1977) reported negative results for point mutations, crossing-over, and non-disjunction. However, Vallini and coworkers (1983) reported positive results for crossing-over and non-disjunction at 30mM. There was a decrease in the response at the higher concentration which the investigators attributed to the growth stimulation effect of the phosphorus in azinphos-methyl on the fungi. Shah *et al.* (1997) reported an increase in DNA adducts (12-fold higher than negative controls) when calf thymus DNA was exposed to metabolically activated azinphos-methyl based on ^{32}P-postlabeling.

E. Conclusions

Azinphos-methyl appears to be genotoxic based on positive results in a mouse lymphoma assay, four *in vitro* cytogenetic assays with human cells or cell lines (primary lymphocytes, WI-38, HEp-2, and L-MOORE cell lines) or Chinese hamster cell line (CHO-K1), and a micronucleus assay with human lymphocytes. However, all of the *in vivo* cytogenetic assays (2 micronucleus assays and 1 cytogenetic assay in mice) were negative. All other tests for chromosomal aberrations, including sister chromatid exchange assays and dominant lethal assays, were negative. Furthermore, most of the reverse mutation assays with *Salmonella typhimurium* were negative except for an equivocal response with TA100 in one assay and a weak positive response in another assay with TA98. The weak positive response was only observed at concentrations (3,333 µg/plate and higher) where precipitation occurred, confounding the results. Negative results were reported for all of the other gene mutation tests and miscellaneous genotoxicity tests, except for a forward mutation assay with *Schizosaccharomyces pombe* ade6, a mitotic recombination assay in *Saccharomyces cerevisiae* D3, a reverse mutation/gene conversion assay with *S. cerevisiae* D7, an assay for gene conversion/crossing-over/non-disjunction in *Aspergillus nidulans* D7, and a ^{32}P-postlabeling assay of adducts in calf thymus DNA.

IX. REPRODUCTIVE TOXICITY

A. Introduction

Three reproductive toxicity studies were available for azinphos-methyl, one in mice and two in rats. In addition, a spermatogenesis study in rabbits was conducted with azinphos-methyl. Only one of the reproductive toxicity studies met FIFRA guidelines.

B. Oral Studies

1. Mice

In a 3-generation, 2-litter study, 24 female and 6 male CF1 mice/group were given azinphos-methyl (80%) in the diet at 0, 5, 10, 25 or 50 ppm (0, 0.075, 1.5, 3.75 or 7.5 mg/kg/day)[10] (Root *et al.*, 1965). The adults were fed the control or treated diet 30 days prior to mating. Thirty-day old F3b pups were sacrificed and submitted for macroscopic and microscopic examination. Nine and 15 pre-mating deaths occurred in the P0 females at 10 and 50 ppm, respectively. The deaths at 10 ppm were not considered compound-related by the investigators because the animals that died had severe diarrhea and other symptoms that were similar to other animals not on the study that had died and the deaths occurred in only two of six cages (the animals were group housed). The investigators concluded that the deaths at 50 ppm were compound-related because they occurred in all six cages of this group. Although fertility was not affected in the surviving mice at 50 ppm, this dose level was discontinued in the subsequent generations due to the high mortality rate. There was no compound-related effect on the fertility and gestation indices or the incidence of macroscopic and microscopic lesions. There was a decrease (66%) in the lactation index (percent of live pups from day 4 that survived until day 21) at 50 ppm. The apparent reproductive and parental NOEL was 25 ppm (3.75 mg/kg/day) based on the reduced survival of offspring and mortalities in adults, respectively. DPR toxicologists found this study unacceptable due to major deficiencies including no individual data, no diet analysis, inadequate group size and inadequate exposure period prior to mating.

2. Rats

In a 2-generation, 2-litter study, azinphos-methyl (87.2%) was administered in the diet at 0, 5, 15, or 45 ppm (F_0M: 0, 0.33, 1.02 or 3.46 mg/kg/day; F_0F: 0, 0.48, 1.48 or 4.84 mg/kg/day; F_1BM: 0, 0,42, 1.22 or 7.37 mg/kg/day; F_1BF: 0, 0.67, 2.02 or 10.27 mg/kg/day) to 12 male and 24 female Bor:WISW (SPF-Cpb) rats/group (Eiben and Janda, 1984). Alopecia (onset week 6), inflammation around eyes (onset week 3), convulsions (onset week 24) and mortality (20%, onset week 5) were observed at 45 ppm. The mean body weights were reduced (9%) in females at 45 ppm. The viability index (percent of pups born live that survived to day 4) and lactation index were reduced 60-68% and 53-72%, respectively, at 45 ppm in both the F_1A and F_1B generations. The viability and lactation indices were also slightly reduced (11 and 8%, respectively) at 15 ppm in one generation, but not both (F_1A - viability index, F_1B – lactation index). ChE activity was not measured in this study, but based on other studies conducted in this

[10] Estimated assuming a 28 g mouse consumes 5 g of feed per day (U.S. EPA, 1988).

laboratory using similar dose levels (Eiben *et al.*, 1983; Schmidt and Chevalier, 1984), the registrant suggested that the reproductive effects were due to significant ChE inhibition occurring at 15 ppm even though no cholinergic signs were observed (Van Goethem, 1987). The mean RBC and brain ChE were reduced (73 and 82% of control activity, respectively) in females at 20 ppm in a 28-day range-finding study (Eiben *et al.*, 1983). Therefore, DPR toxicologists lowered the parental NOEL from 15 to 5 ppm (F_0M: 0.33 mg/kg/day; F_0F: 0.48 mg/kg/day; F_1BM: 0.42 mg/kg/day; F_1BF: 0.67 mg/kg/day) based on the ChE inhibition data from these other studies. The reproductive NOEL was also 5 ppm based on the decreased viability and lactation indices. This study was considered acceptable to DPR toxicologists based on FIFRA guidelines.

Eighteen male and 46 female Wistar derived (Bor:WISW; SPF Cpb) rats/group were fed azinphos-methyl (91.7%) in the diet at 0, 5, 15 or 45 ppm (M: 0, 0.43, 1.30 or 3.73 mg/kg/day; F: 0, 0.55, 1.54 or 4.87 mg/kg/day during premating period) for one generation (Holzum, 1990). Ten additional males/group were mated with 20 untreated females. The mean body weights were slightly reduced (<10%) in both sexes at 45 ppm of the F_0 generation during several weeks of the mating period. Five females at 45 ppm died without clinical signs during weeks 3 and 6 of mating. Two other females at 45 ppm were sacrificed in a moribund condition in weeks 3 and 10 after exhibiting poor general condition, inertia, nasal discharge, and stumbling gait. Hyperemia and edema of the lungs and centrilobular hyperemia of the liver were observed histologically in the animals that died or were moribund. The investigators attributed these deaths to non-homogeneous mixing of the diets which occurred during weeks 3, 4 and 6 of mating. There was no effect on food consumption, insemination index, fertility index, gestation index, gestation period, lactation index, or clinical signs of pups. The viability index and pup body weights during the lactation period were significantly reduced (8-48% and 14-23%) at 15 and 45 ppm, respectively. At sacrifice, the mean plasma ChE activity was reduced at 15 ppm (M: 86%; F: 61% of controls) and 45 ppm (M: 57%; F: 37% of controls) of the F_0 generation. The mean RBC ChE activity was significantly depressed at 5 ppm (M: 81%; F: 53% of controls), 15 ppm (M: 31%; F: 16% of controls), and 45 ppm (M: 6%; F: 11% of controls) in the F_0 generation. The mean parental brain ChE activity was also reduced at 15 ppm (F: 52% of controls) and 45 ppm (M: 81%; F: 32% of controls). The mean brain ChE activity in pups was only reduced at 45 ppm (54% of controls) on postpartum day 28. The parental NOEL for overt toxicity was 5 ppm (M: 0.43 mg/kg/day; F: 0.55 mg/kg/day) based on the brain ChE inhibition. The parental NOEL for RBC ChE inhibition appears to be was less than 5 ppm. The reproductive NOEL was also 5 ppm based on the decreased viability index and pup weight. This study was considered supplemental by DPR toxicologists, supporting the conclusions in the previous study that reduction in certain reproductive parameters occurs at the same dose level that significant ChE inhibition occurs. However, it does not establish a definitive link between the reproductive effects and the maternal toxicity.

3. Rabbits

Spermatogenesis was examined in a study where 20 sexually mature male Buscat rabbits were administered azinphos-methyl orally by gavage at 1.5 mg/kg/day for 12 weeks (Soliman

and El-Zalabani, 1981). An additional 10 male rabbits of comparable age served as controls. There was no effect on semen volume, but there was a significant decrease (42%) in mean sperm count and a significant increase (169%) in mean percent of abnormal spermatozoa. The testes in all treated rabbits exhibited varying degrees of impaired spermatogenesis when examined histologically. The histological changes included reduced size of seminiferous tubules with "a consequent increase in intertubular fibrous tissue stroma", a decrease in the number of all germ cells, degeneration and necrosis in the seminiferous tubules. Spermatogenesis was arrested primarily at the spermatid level. The Leydig and Sertoli cells appeared normal. Due to the limited endpoints examination, and only one dose level tested, a NOEL could not be established for this supplemental study.

C. Conclusions

There were a variety of adverse effects observed in the parental animals in the reproductive toxicity studies including deaths, alopecia, inflammation around eyes, convulsions, ataxia, nasal discharge, lethargy, reduced body weights, ChE inhibition, hyperemia and edema of the lungs, and centrilobular hyperemia of the liver. The lowest NOEL for overt parental toxicity was 5 ppm (F_0M: 0.33 mg/kg/day; F_0F: 0.48 mg/kg/day; F_1BM: 0.42 mg/kg/day; F_1BF: 0.67 mg/kg/day) based on brain ChE inhibition. The parental NOEL for plasma and RBC ChE inhibition was less than 5 ppm. Several reproductive effects were also noted including a reduction in the lactation and viability indices, reduced pup weights, reduced sperm count, increased number of abnormal spermatozoa, reduced size, degeneration and necrosis of the seminiferous tubules and decreased number of germ cells in the testes. The lowest NOEL for reproductive effects was also 5 ppm based on a reduction in viability and lactation indices.

X. DEVELOPMENTAL TOXICITY

A. Introduction

Seven developmental toxicity studies were available for azinphos-methyl including two in mice, two in rats and three in rabbits. Only one rat and one rabbit study were acceptable based on the FIFRA guidelines.

B. Oral Studies

1. Mice

Groups of 22-23 pregnant CD-1 mice were administered technical grade azinphos-methyl (purity not stated) in corn oil by gavage at 0, 1.25, 2.5, and 5 mg/kg/day from gestation day 6 to 15 and sacrificed on day 18 (Short *et al.*, 1978). Cholinergic signs (salivation, urination, tremors) and death were observed in the dams at 5 mg/kg/day. The time of onset of these signs was not reported. There was no effect on litter size, incidence of resorptions, fetal body weights, external or soft tissue anomalies at any dose level. A significant increase in the incidence of malaligned sternebrae was observed at 5 mg/kg/day. The average percent of fetuses per litter with malaligned sternebrae were 6.4 and 24.3 at 0 and 5 mg/kg/day, respectively. The apparent maternal and developmental NOEL was 2.5 mg/kg/day based on cholinergic signs and malaligned sternebrae, respectively. However, DPR toxicologists found this study unacceptable due to major deficiencies including no individual data, purity information or analyses of dosing solutions.

Azinphos-methyl (purity not reported) was administered to 15, 20 and 40 CD-1 pregnant female mice at 0, 16 and 20 mg/kg, respectively, by gavage in corn oil on day 8 of gestation (Kavlock et al., 1985). One dam at 16 mg/kg and 21 dams at 20 mg/kg died. The mean maternal weight gain was reduced by 6 and 20% at 16 and 20 mg/kg, respectively, but was not statistically significant at either dose level. A reduction in the mean fetal weight (11%) was observed at 20 mg/kg. A significant increase in supernumerary ribs (extra ribs) was observed at both dose levels. The investigators suggested that the increase in extra ribs was not treatment-related, but rather was related to reduced maternal weight gain. They found a significant inverse relationship ($p < 0.001$) between maternal weight gain and extra ribs when they combined data for 10 unrelated chemicals (cacodylic acid, caffeine, deltamethrin, dinoseb, ethylene bisisothiocyanate sulfide, endrin, azinphos-methyl, kepone, sodium salicylate, and toxaphene). DPR toxicologists did not concur with the investigators and assumed that the extra ribs were treatment-related. Therefore, the developmental NOEL was assumed to be less than 16 mg/kg based on the extra ribs. The maternal NOEL also was less than 16 mg/kg based on one mortality and slightly reduced weight gain. This study had major deficiencies including only one day exposure and no maternal clinical signs or gross pathology data.

2. Rats

Charles River CD rats (21 pregnant rats/dose) were administered azinphos-methyl (purity not reported) in corn oil by gavage at 0, 1.25, 2.5 or 5 mg/kg/day during gestation days 6-15 (Short *et al.*, 1978). An additional 14-15 pregnant rats/dose were administered azinphos-methyl at the same dose levels from day 6 of gestation until the pups were weaned on day 21. Pups were sacrificed at 30 to 40 days of age. Cholinergic signs (tremors, salivation, urination) and death were observed in the dams at 5 mg/kg/day. The time of onset of these signs was not reported. A reduction in the mean maternal body weight gain and food consumption was also noted (52% and 24%, respectively, during the exposure period). There was no effect on litter size, incidence of resorptions, fetal body weight or external, visceral or skeletal anomalies. The developmental NOEL was equal to or greater than 5 mg/kg/day, the highest dose tested. The maternal NOEL was 2.5 mg/kg/day based on the cholinergic signs, reduced maternal weight gain, and reduced food consumption. This study was unacceptable to DPR toxicologists due to major deficiencies including no individual data, purity information or analyses of dosing solutions.

Azinphos-methyl (87.7%) was given in a 6% Emulphor emulsion by gavage to 33 pregnant Charles River Crl:CD BR rats/dose at 0, 0.5, 1.0, or 2.0 mg/kg on days 6-15 of gestation (Kowalski *et al.*, 1987). Five rats/dose were sacrificed on day 16 of gestation and 28 rats/dose on day 20. The dams exhibited no clinical signs at any dose level, although the mean plasma, RBC and brain ChE activity were significantly reduced in the 2.0 mg/kg/day dams on day 16 (63%, 77%, and 61% of control activity, respectively). By day 20, only the mean brain ChE activity was still significantly reduced (73% of control activity). The mean brain ChE activity in the fetuses was not reduced even at 2.0 mg/kg/day. There was also no evidence for developmental toxicity at any dose. Therefore, the developmental NOEL was greater than or equal to 2.0 mg/kg/day, the highest dose tested. The maternal NOEL was 1.0 mg/kg/day based on the plasma, RBC and brain ChE inhibition. DPR toxicologists found this study acceptable.

3. Rabbits

Ten pregnant New Zealand white female rabbits/group were administered azinphos-methyl (92.7%) in the diet at 0, 5 or 25 ppm (0, 0.15 or 0.75 mg/kg/day)[11] on days 8-16 of gestation (Doull *et al.*, 1966). Five females/group were sacrificed on gestation day 29 and the fetuses removed, weighed, and examined for skeletal and visceral anomalies. The other 5 females in each group were allowed to deliver and nurse their pups until lactation day 30. The pups were then examined for gross pathological effects. There was no effect on the fertility index, litter size, survival of offspring, and gross pathological findings in the fetuses. The maternal and developmental NOELs appear to be equal to or greater than 25 ppm (0.75 mg/kg/day), the highest dose tested. DPR toxicologists considered this study unacceptable due to numerous deficiencies including no diet analysis, inadequate group size, inadequate exposure period, no body weight or food consumption data, and no individual data.

11 Estimated assuming a 4.0 kg New Zealand White rabbit consumes 120 g of feed per day (U.S. EPA, 1988).

Azinphos-methyl (92.4%) was administered in a 0.5% Cremophor emulsion by gavage to 9-11 pregnant female Himalayan rabbits/dose at 0, 0.3, 1 or 3 mg/kg/day on gestation days 6-18 (Machemer, 1975). There was no evidence of maternal toxicity (mortality, clinical signs, weight gain) or developmental toxicity (increased number of resorptions or abortions, reduced litter size or fetal weight, alteration in the sex ratio, external, brain or skeletal malformations). The maternal and developmental NOELs were equal to or greater than 3 mg/kg/day, the highest dose tested. DPR toxicologists found this study unacceptable due to major deficiencies including lack of maternal toxicity at the highest dose, and missing data on uterine weights, corpora lutea and resorptions.

A teratology study was also performed using 17-18 artificially inseminated American Dutch female rabbits given azinphos-methyl in a 7% Emulphor emulsion by gavage at 0, 1, 2.5 or 6 mg/kg/day on days 6-18 of gestation (Clemens *et al.*, 1988). Ataxia and tremors (onset day 16) were observed in 4 does at 6 mg/kg/day. On day 19, the mean maternal plasma ChE activity was significantly lower at 2.5 and 6 mg/kg/day (87% and 78% of controls, respectively). The mean RBC ChE activity was reduced at 1, 2.5 and 6 mg/kg/day (86%, 80% and 50% of controls, respectively), although only the reduction at 6 mg/kg/day was statistically significant. On day 28, the mean maternal RBC and brain ChE activities were reduced only at 6 mg/kg/day (87% and 88% of control activity, respectively), but the reduction in RBCs was not statistically significant. There was a significant decrease in litter size at 6 mg/kg/day apparently due to pre- and post-implantation losses (Table 14). The median pre-implantation loss was significantly higher at 1, 2.5, and 6 mg/kg/day. However, the investigators indicated that the pre-implantation loss was

Table 14. Developmental Effects in Rabbits Exposed to Azinphos-methyl[a]

		Dose Level (mg/kg/day)			
		0	1	2.5	6
Litter size	mean	7.4	6.2	7.0	5.5
	median	7.0	7.0	7.0	6.0*
	(range)	(4-10)	(1-9)	(3-11)	(2-8)
% Pre-implantation loss	mean	1.5	23.0	14.8	28.0
	median	0.0	11.3**	12.5*	30.3**
	(range)	(0-13)	(0-78)	(0-50)	(0-60)
% Post-implantation loss	mean	2.4	3.0	4.3	7.2
	median	0.0	0.0	0.0	0.0
	(range)	(0-20)	(0-25)	(0-29)	(0-33)
Median weight of live fetuses	male	36.7	37.9	35.2	40.1**
(grams)	female	35.9	36.2	35.7	38.2
	(combined)	37.1	38.2	36.1	39.4**
Median weight of placentas	(grams)	5.4	5.4	5.1	6.0*

[a] Does exposed from days 6-18 of gestation

*, ** Significantly different from controls at $p < 0.05$ and 0.01, respectively, by the Kruskal Wallis test.

within the historical control range (0-13.3%) at 1 and 2.5 mg/kg/day. There was also a slight increase in the mean post-implantation loss, but the difference was not statistically significant. The median weight of live fetuses and placentas were also significantly higher at 6 mg/kg/day, possibly due to the smaller litter size. The maternal NOEL for overt toxicity was 2.5 mg/kg/day based on the clinical signs and brain ChE inhibition. The maternal NOEL for RBC ChE inhibition was less than 1.0 mg/kg/day. The developmental NOEL was also 2.5 mg/kg/day based on the increased pre- and post-implantation losses. This study was acceptable to DPR toxicologists.

C. Conclusions

Evidence of maternal toxicity was observed in all three species. Cholinergic signs including salivation, urination, tremors and ataxia were seen. Other maternal effects included reduced body weight gains, reduced food consumption, ChE inhibition and death. The lowest maternal NOEL for overt toxicity in an acceptable study was 1.0 mg/kg/day based on brain ChE inhibition in rats. The lowest NOEL for blood ChE inhibition was less than 1 mg/kg/day based on RBC ChE inhibition in rabbits. Several developmental effects were also seen including malaligned sternebrae, extra ribs, increased pre- and post-implantation losses and reduced pup weight. The lowest developmental NOEL in an acceptable study was 2 mg/kg/day, the highest dose tested in rats.

XI. NEUROTOXICITY

A. Introduction

Five delayed neurotoxicity studies in hens were available for azinphos-methyl, two acute studies and three subchronic studies. Only one acute study was found acceptable to DPR toxicologists based on the FIFRA guidelines. Two behavioral neurotoxicity studies in rats were also available, one acute study and one subchronic study. Both studies met FIFRA guidelines.

B. Acute

1. Oral Studies

a. Hens

White leghorn hens were administered a single dose of azinphos-methyl (purity not reported) at 1-250 mg/kg without delayed neurotoxic effects (Kimmerle and Löser, 1974). The NOEL for delayed neuropathy was equal to or greater than 250 mg/kg, the highest dose tested. This published report was not submitted to DPR for review.

Thirty white leghorn hens were administered azinphos-methyl (85%) by gavage at 330 mg/kg with atropine (15 mg/kg) administered intramuscularly 15 minutes prior to dosing (Glaza, 1988). This treatment was repeated 21 days later. No clear evidence of delayed neuropathy was observed during the 44 day observation period. DPR toxicologists found this study acceptable.

b. Rats

Groups of 18 Fischer 344 rats/sex/dose were evaluated for neurotoxic effects after receiving a single dose of azinphos-methyl (92.2-92.8% purity) by oral gavage at 0, 2, 6 or 13 mg/kg for males and 0, 1, 3 or 6 mg/kg for females (Sheets, 1994). Twelve rats/sex/dose were assigned to the main study and 6 rats/sex/dose were assigned to a satellite group for ChE determination. Five males at 13 mg/kg and 15 females at 6 mg/kg died on the day of dosing. Most of these animals died before clinical observations were done. One surviving female at 6 mg/kg had oral and urine stains. Surviving males at 13 mg/kg had muscle fasciculations, tremors, gait incoordination, and oral/nasal/urine stains. No compound-related signs were observed in females at 3 mg/kg; however, males at 2 mg/kg had muscle fasciculations and oral stains. The onset of these signs was on day 0, and they were resolved by day 3. The functional observational battery (FOB) was conducted 30 minutes to 1 hour after dosing. Due to the early deaths, only 11 males and 3 females at the high-dose level were available for the FOB. In the FOB on Day 0, animals of both sexes exhibited various neurobehavioral changes at the mid- and high-dose levels (Table 15). The effects in females at 3 mg/kg were not statistically significant; however, given that the majority (15/18) of females at 6 mg/kg died before the FOB could be conducted these effects were considered biologically significant. Reductions of 43% and 77% in session motor and locomotor activity, respectively, were seen in males at 13 mg/kg. Females at 6 mg/kg showed similar reductions (45% and 63%) in motor and locomotor activity. The

Table 15. Neurobehavioral Changes in Rats on Day 0 After a Single Oral Dose of Azinphos-methyl by Oral Gavage[a]

Parameter	Dose Level (mg/kg)			
Males	**0**	**2**	**6**	**13**
Functional Observational Battery				
Lacrimation	0 (0%)[b]	0 (0%)	3 (25%)	1 (8%)
Salivation	0 (0%)	0 (0%)	4 (33%)	4 (33%)
Repetitive Chewing	0 (0%)	0 (0%)	8 (67%)*	10 (83%)*
Muscle Fasciculations	0 (0%)	0 (0%)	12 (100%)*	9 (75%)*
Tremors	0 (0%)	0 (0%)	6 (50%)*	9 (75%)*
Uncoordinated Gait	0 (0%)	0 (0%)	6 (50%)*	7 (58%)*
Sitting or Lying	0 (0%)	0 (0%)	3 (25%)*	6 (50%)*
Reduced Approach Response	6 (50%)	6 (50%)	11 (92%)	10 (83%)
Reduced Touch Response	1 (8%)	1 (8%)	5 (42%)*	6 (50%)*
Uncoordinated Righting Reflex	2 (17%)	1 (8%)	8 (67%)*	9 (75%)*
Body Temperature	37.8±0.3[c]	37.9±0.3	36.5±0.9*	36.3±0.9*
Grip Strength, Forelimb	0.83±0.07	0.82±0.08	0.71±0.18	0.57±0.31*
Grip Strength, Hindlimb	0.50±0.06	0.47±0.06	0.41±0.07*	0.35±0.12*
Activity				
Motor	176±42	208±75	112±81	100±107
Locomotor	61±13	68±26	32±21	14±13
Females	**0**	**1**	**3**	**6**
Functional Observational Battery				
Lacrimation	0 (0%)	0 (0%)	0 (0%)	1 (33%)*
Salivation	0 (0%)	0 (0%)	0 (0%)	1 (33%)*
Repetitive Chewing	0 (0%)	0 (0%)	0 (0%)	1 (33%)*
Muscle Fasciculations	0 (0%)	0 (0%)	0 (0%)	1 (33%)*
Tremors	0 (0%)	0 (0%)	0 (0%)	1 (33%)*
Uncoordinated Gait	0 (0%)	0 (0%)	0 (0%)	1 (33%)*
Sitting or Lying	0 (0%)	0 (0%)	1 (8%)	1 (33%)*
Reduced Approach Response	1 (8%)	2 (17%)	5 (42%)	2 (67%)
Reduced Touch Response	0 (0%)	0 (0%)	0 (0%)	1 (33%)
Uncoordinated Righting Reflex	1 (8%)	1 (8%)	3 (25%)	2 (33%)
Body Temperature	38.1±0.2	38.1±0.3	37.9±0.6	37.0±1.7
Grip Strength, Forelimb	0.73±0.06	0.74±0.09	0.73±0.09	0.53±0.33*
Grip Strength, Hindlimb	0.36±0.06	0.34±0.05	0.37±0.08	0.32±0.06*
Activity				
Motor	245±136	198±104	196±106	135±101
Locomotor	79±40	58±20	67±41	29±18

[a] Sheets, 1994

[b] Incidence per 12 animals, except in females at 6 mg/kg where only 3 survivors were tested; number in parentheses represents the incidence in percentage.

[c] Mean ± standard deviation

* Significantly different from control group ($p < 0.05$) by analysis of contrasts for categorical data and by Dunnett's test for continuous data.

reductions in motor and locomotor activity were not statistically significant in either sex at any dose level, due in part to the high mortality of females at 6 mg/kg and the variability in males at 6 or 13 mg/kg. The investigators suggested these reductions were biologically significant based on a general standard of 20% difference from control.

Blood and brain samples were collected for ChE measurements approximately 90 minutes after dosing. Due to the early death of all of the females in the satellite group at 6 mg/kg, no samples were collected from this group. The mean plasma and RBC ChE activity was reduced in males at all dose levels (Table 16). The mean brain ChE activity was only reduced at 6 and 13 mg/kg. In females, only the mean RBC ChE activity was reduced at all dose levels. The mean plasma and brain ChE activity were only reduced at 3 mg/kg. No dose-related macroscopic, microscopic or organ weight changes were found. The NOEL for overt neurotoxic effects was 1 mg/kg based on the effects observed in the FOB (sitting or lying in open field, reduced approach response and uncoordinated righting response) and brain ChE inhibition (49% of controls) in females. The NOEL for RBC ChE inhibition was less than 1 mg/kg in females. This study was acceptable to DPR toxicologists.

Table 16. Cholinesterase Activity in Plasma, Red Blood Cells and Brain of Rats 90 Minutes After a Single Dose of Azinphos-methyl by Oral Gavage[a]

Tissue	**Dose Level (mg/kg)**		
Males	**2**	**6**	**13**
Plasma	68%[b]*	43%*	50%*
RBCs[c]	67%*	33%*	37%*
Brain	85%	26%*	12%*
Females	**1**	**3**	**6**
Plasma	89%	64%*	---
RBCs	83%*	35%*	---
Brain	95%	49%*	---

[a] Sheets, 1994.
[b] Percent relative to control activity. Six animals examined per sex per dose level.
[c] RBCs = red blood cells
* Significantly different from controls ($P < 0.05$) by the Dunnett's test.

C. Subchronic

1. Oral Studies

a. Hens

Eight HNL chickens/dose were fed azinphos-methyl (80%) in the diet at 0, 75, 150, 300 or 600 ppm for 30 days followed by a 4-week observation period (Kimmerle, 1964). A slight reduction in the mean whole blood ChE activity (73-84% of control activity) was observed in animals at 300 and 600 ppm at the end of the exposure period. No clinical signs and only a slight weight reduction were observed in the chickens at 600 ppm. DPR toxicologists found the study

unacceptable due to insufficient information regarding adverse effects, missing data on clinical observations and histopathology, no positive control and inappropriate route of administration.

Groups of 6 Leghorn hens/dose were fed azinphos-methyl (purity not stated) in the diet at 0, 10, 50 or 100 ppm for 30 days and observed for an additional 30 days (Tayler, 1965). No abnormal clinical signs or histological evidence of demyelination was observed. This study was unacceptable to DPR toxicologists due to the lack of individual data, no positive control and inappropriate route of administration.

In a repeat experiment, 8 HNL chickens/dose were fed azinphos-methyl (80%) in the diet at 0, 900, 1200, 1500 or 1800 ppm for 30 days followed by a 4-week observation period (Kimmerle, 1965). No whole blood ChE inhibition was observed at any dose level. There was a slight reduction in the mean body weights in all treatment groups (4-15%) during the exposure period. No other overt signs of toxicity were noted. DPR toxicologists found this study unacceptable due to major deficiencies (no individual data for clinical signs and histological observations, no positive control, inappropriate route of administration).

b. Rats

Azinphos-methyl (92.2% purity) was fed to 18 Fischer 344 rats/sex/dose in the diet at 0, 15, 45 or 120 ppm for males (0, 0.91, 2.81 or 7.87 mg/kg/day) and at 0, 15, 45 or 90 ppm for females (0, 1.05, 3.23 or 6.99 mg/kg/day) for 13 weeks (Sheets and Hamilton, 1995). Twelve rats/sex/dose were used for neurobehavioral observation with half also undergoing neuropathological examination. The remaining 6 rats/sex/dose were used for ChE determinations only. Increased reactivity, perianal stain, red lacrimation, and oral stain were observed in males at 120 ppm and in females at 45 and 90 ppm. In addition, females at 90 ppm had uncoordinated gait and tremors. These clinical signs were observed within the first few weeks of exposure and persisted with continued exposure. The body weights and food consumption were reduced in males at 120 ppm (9-10%) and in females at 90 ppm (15-45%). The food consumption was reduced only during the first few weeks. In the FOB, perianal/urine stain was the only sign observed in males at 120 ppm and in females at 45 ppm from weeks 4 through 13 (Table 17). Urine stain, increased reactivity, decreased forelimb grip strength, impaired righting reflex, and tremors were observed in the females at 90 ppm at week 4. Only the increased reactivity, urine stain and reduced forelimb grip strength were still present at week 13. Motor and locomotor activity were significantly reduced (33-60%) in males at 120 ppm at weeks 4, 8 and 12 and in females at 90 ppm at week 4. ChE activity was significantly reduced at all dose levels for both sexes in plasma, RBC, and brain (Table 18). There was no treatment-related effect on mortalities, ophthalmic findings, macroscopic or microscopic lesions, or brain weights. The NOEL was less than 15 ppm (M: 0.91 mg/kg/day; F: 1.05 mg/kg/day) based on the plasma, RBC and brain ChE inhibition in both sexes. DPR toxicologists found this study acceptable.

Table 17. Neurobehavioral Changes in Rats at Week 4 in a Subchronic Oral Neurotoxicity Study[a]

Parameter	Dose Level (ppm)			
Males	**0**	**15**	**45**	**120**
Functional Observational Battery				
Stains, Perianal	0 (0%)[b]	0 (0%)	0 (0%)	4 (33%)
Activity				
Motor	482±119[c]	415±146	449±155	241± 81*
Locomotor	178± 54	165± 63	167± 57	77± 21*
Females	**0**	**15**	**45**	**90**
Functional Observational Battery				
Increased Reactivity	0 (0%)	0 (0%)	0 (0%)	6 (50%)*
Stains, Urine	1 (8%)	1 (8%)	3 (25%)	11 (92%)*
Tremors	0 (0%)	0 (0%)	0 (0%)	5 (42%)*
Uncoordinated Righting Reflex	3 (25%)	1 (8%)	2 (17%)	8 (67%)*
Grip Strength, Forelimb	0.63±0.09	0.63±0.07	0.65±0.08	0.47±0.06*
Activity				
Motor	1038±410	996±332	816±256	460±170*
Locomotor	384±172	375±125	335±112	154± 63*

[a] Sheets and Hamilton, 1995

[b] Incidence per 12 animals; percentage affected in parentheses.

[c] Mean ± standard deviation

* Significantly different from control group ($p < 0.05$) by analysis of contrasts for categorical data and by Dunnett's test for continuous data.

D. Conclusions

There was no evidence of delayed neuropathy after acute or subchronic exposure to azinphos-methyl. Neurobehavioral changes were seen in rats after acute and subchronic exposure to azinphos-methyl. The neurobehavioral effects included increased reactivity, repetitive chewing, muscle fasciculations, tremors, gait incoordination, lacrimation, salivation, perianal/urine stains, reduced approach and touch responses, reduced righting reflexes, reduced body temperature, reduced grip strength and reduced motor and locomotor activity. In addition, significant reductions in the ChE activity in the plasma, RBCs and brain were observed. The acute NOEL for overt neurotoxic effects was 1 mg/kg based on reduced activity, reduced approach response and uncoordinated righting response and brain ChE inhibition (49% of controls) in females. The acute NOEL for RBC ChE inhibition was less than 1 mg/kg in females. The subchronic NOEL was less than 15 ppm (M: 0.91 mg/kg/day; F: 1.05 mg/kg/day) based on reduced plasma, RBC and brain ChE activity in both sexes.

Table 18. Cholinesterase Activity in Plasma, Red Blood Cells and Brain of Rats Fed Azinphos-methyl for 13 Weeks[a]

Tissue	Dose Level (ppm)		
Males	**15**	**45**	**120**
Week 4			
Plasma	93%[b]	58%*	25%*
RBCs[c]	63%*	12%*	2%*
Week 13			
Plasma	85%*	56%*	31%*
RBCs	63%*	16%*	5%*
Brain	92%*	54%*	18%*
Females	**15**	**45**	**90**
Week 4			
Plasma	86%*	41%*	17%*
RBCs	59%*	22%*	9%*
Week 13			
Plasma	87%	40%*	19%*
RBCs	62%*	22%*	5%*
Brain	84%*	28%*	15%*

[a] Sheets and Hamilton, 1995.

[b] Percent relative to control activity. Six animals examined per sex per dose level.

[c] RBCs = red blood cells

* Significantly different from controls ($P < 0.05$) by the Dunnett's test.

XII. IMMUNOTOXICITY

There were no FIFRA guideline immunotoxicity studies available for azinphos-methyl; however, there was one study in the open literature which reported immunological changes after exposure to azinphos-methyl. Six male weanling Wistar-derived rats per group were administered azinphos-methyl (85%) in the diet at 0, 5, 25, or 125 mg/kg/day for 3 weeks (Vos *et al.*, 1983). Several general toxicological and immunological changes were observed at 125 mg/kg/day including increased mortality rate, decreased body weight, decreased relative spleen, pituitary, and mesenteric lymph node weights, and unspecified histopathological changes in the thymus, pituitary, adrenal glands, and testes. It is unclear if the immunological changes are due to azinphos-methyl acting directly on the tissue or indirectly through "stress" (Pruett *et al.*, 1993; Vogel, 1993). Since only a few endpoints were examined, a NOEL could not be established for this study.

XIII. RISK ASSESSMENT

A. Introduction

The risk assessment process consists of four basic elements: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and characterizes the relationship between the amount of exposure (or dose) and the severity or probability of a toxic effect. The amount of exposure which will not likely result in an observable or significant adverse health effect is estimated. In this risk assessment, the exposure assessment includes an estimation of the potential exposure through offsite or ambient air on an acute, subchronic, and chronic basis. Risk characterization then extrapolates the toxic effects observed in the laboratory studies, in which animals are exposed to high dosages of pesticide, to potential human exposures at the low dosages of pesticide residues in ambient air.

B. Hazard Identification

1. Acute Toxicity

The adverse effects observed with the acute studies are summarized in Table 19. In general, the effects that are considered adverse include clinical signs, reductions in body weight and food consumption greater than 10%, and increases in gross and histopathological lesions. Changes in clinical chemistry and hematology values and organ weights without accompanying functional or structural changes are generally not considered adverse. In general, DPR considers brain ChE inhibition to be indicative of overt toxicity since it is one of the primary target sites and more subtle neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC ChE inhibition is less certain because the ChEs in blood have no known physiological function. As mentioned in the Introduction, plasma ChE, or more specifically butyrylcholinesterase (BuChE), may be involved in the binding/metabolism of certain drugs, such as succinylcholine, which suggests that its inhibition may compromise an organism's ability to defend against subsequent toxic insults. BuChE is also the predominant form of ChE in the developing nervous system of birds and mammals (Brimijoin and Koenigsberger, 1999). U.S. EPA does not consider plasma or RBC ChE inhibition an adverse effect in itself, but does use it as a surrogate for peripheral ChE inhibition (Sette, 1998). However, it is unclear how representative plasma or RBC ChE activity is of peripheral ChE activity. Plasma ChE is primarily BuChE which is a different enzyme than acetylcholinesterase (AChE) that is involved in neurotransmission. As a result, ChE inhibitors can have different affinities for the active sites of BuChE and AChE. The ChE in RBCs is AChE, but RBCs lack the ability to synthesize new AChE (Brimijoin, 1992). The recovery of RBC ChE activity is dependent on the replacement of RBCs, and, consequently, is much slower than in neurological and neuromuscular tissue. The Joint Meeting on Pesticide Residues of the FAO/WHO concluded only RBC ChE activity at the time of peak effect with acute exposure should be used as a surrogate for peripheral ChE activity (JMPR, 1999).

Table 19. Acute Effects of Azinphos-Methyl and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL (mg/kg)	LOEL (mg/kg)	Ref.[a]
		Inhalation			
Rat[b]	Single, 1-hr	Unspecified signs of toxicity	2.7[c]	8.9	1
	Single, 4-hr	Unspecified signs of toxicity	4.1	10.5	
Rat[b]	Single, 4-hr	Cholinergic signs	-----	17.8[d](M) 14.4 (F)	2*
		Oral			
Rat[b]	Single, gavage	Unspecified signs of toxicity	2.5	5.0	3
Rat[b]	Single, gavage	Cholinergic signs	-----	2.0	4
Rat[b]	Single, gavage	Cholinergic signs	-----	4.0	5
Rat[b]	Single, gavage	Cholinergic signs	1.0	2.5	6
Rat[b]	Single, gavage	Cholinergic signs	-----	5.0	7
Rat[e]	**Single, gavage**	**Inactivity, red. reflexes, plasma and brain ChE[f] inhibition (F: 49-64%)[g]**	**1.0**	**3.0**	**8***
		RBC[h] ChE inhibition (F: 83%)	**(0.1)[i]**	**1.0**	
Human	**Single, capsule**	**Plasma and RBC ChE inhibition**	**0.75**	**-----**	**9**
Mouse[j]	Single, gavage	Maternal: Death, reduced weight gain	-----	16.0	10
		Fetal: Extra ribs	-----	16.0	
Mouse[j]	9 Days, gavage	Maternal: Cholinergic signs, Death[k]	2.5	5.0	11
		Fetal: Malaligned sternebrae	2.5	5.0	
Rat[j]	9 Days, gavage	Maternal: Cholinergic signs, Death[k]	2.5	5.0	
Rabbit[j]	12 Days, gavage	Fetal: Increased pre- and post- implantation losses	2.5	6.0	12*
		Dermal			
Rat[b]	Single, 24 hrs	Cholinergic signs	-----	100	6
Rat[b]	Single, 24 hrs	Cholinergic signs	----- -----	100 (M) 63 (F)	7

a References: 1. Kimmerle, 1966; 2. Shiotsuka, 1987; 3. Hecht, 1955; 4. Crawford and Anderson, 1974; 5. Lamb and Anderson, 1974; 6. Mihail and Lorke, 1978; 7. Heimann, 1982; 8. Sheets, 1994; 9. MacFarlane and Freestone, 1998; 10. Kavlock *et al.*, 1985; 11. Short *et al.*, 1978; 12. Clemens *et al.*, 1988.

b LD_{50}/LC_{50} study

c Assuming a male Wistar rat weighs 215 g and breathes 0.0096 liters per hour (U.S. EPA, 1988)

d Assuming a male Sprague Dawley rat weighs 265 g and breathes 0.045 m^3 in 4 hours; a female Sprague Dawley rat weighs 204 g and breathes 0.037 m^3 in 4 hours (U.S. EPA, 1988)

e Neurobehavioral study

f ChE = cholinesterase

g Percent of control activity

h RBC = red blood cell

i Estimated by dividing LOEL by a default uncertainty factor of 10 (Dourson and Stara, 1983).

j Developmental toxicity study: All fetal effects were considered acute effects; however, only maternal effects observed within the first few days of exposure were considered acute exposure.

k The time of onset of the maternal effects was not reported; therefore, it was assumed they occurred within the first few days.

* Acceptable study based on FIFRA guidelines

For acute exposure, some effects observed in the developmental toxicity studies were also included. These include maternal effects observed within the first few days of exposure and all fetal effects. Fetal effects were observed in several developmental toxicity studies for azinphos-methyl including extra ribs in fetal mice at 16 mg/kg, malaligned sternebrae in fetal rats at 5 mg/kg and embryotoxicity (increased pre- and post-implantation losses) in rabbits at 6 mg/kg (Kavlock *et al.,* 1985; Short *et al.,* 1978; Clemens *et al.,* 1988). These effects were seen at doses that produced maternal toxicity, although sometimes the maternal effects were not considered acute effects based on their onset. Among the developmental toxicity studies, only one rat and one rabbit study did not have major deficiencies.

Cholinergic signs were the primary effects observed in adult animals in the acute studies for azinphos-methyl with the LOELs generally between 2-6 mg/kg. However, these studies, like most of the acute LD_{50}/LC_{50} studies, had major deficiencies such as an inadequate description of clinical signs observed at each dose level and the dose levels were too high to establish a NOEL. An acceptable acute neurotoxicity study in rats was selected as the definitive study for acute toxicity for azinphos-methyl in laboratory animals (Sheets, 1994). The critical NOEL for overt toxicity was 1 mg/kg based on neurobehavioral effects in females in the functional observational battery (sitting/lying in open field, reduced approach response and uncoordinated righting response) and brain ChE inhibition (49% of controls). The NOEL for blood ChE inhibition in this study was less than 1 mg/kg/day, the lowest dose level tested, based on the RBC ChE inhibition (83% of controls) in females. The critical NOEL for RBC ChE inhibition in rats was estimated to be 0.1 mg/kg by dividing the LOEL by the default uncertainty factor of 10 (Dourson and Stara, 1983).

No statistically significant plasma or RBC ChE inhibition was observed in human volunteers given a single capsule containing azinphos-methyl at the highest dose levels tested, 0.75 and 1.0 mg/kg in females and males, respectively (MacFarlane and Freestone, 1998). No treatment-related clinical signs or symptoms were seen at any dose level. Volunteers were not subjected to any neurobehavioral or neurophysiological testing to evaluate for more subtle neurological effects in cognition or nerve conduction. However, given that no significant plasma or RBC ChE inhibition was seen, no neurological effects would be anticipated based on the neurotoxicity data for azinphos-methyl in animals. DPR has no requirement for human testing of pesticides and there are no FIFRA guidelines for this type of study. However, the study was conducted in a double-blind manner following AGood Clinical Practices@ guidelines and had an extensive informed consent form. Subjects were free to leave the study at any time and were paid in full if they left for health reasons. Therefore, this study was selected as the definitive study for acute toxicity in humans with a critical NOEL of 0.75 mg/kg for plasma and RBC ChE inhibition.

2. Subchronic Toxicity

The effects observed in laboratory animals after subchronic exposure to azinphos-methyl are summarized in Table 20**.** Included in this table are four standard subchronic toxicity studies: one inhalation study with rats, two oral studies with rats and one dermal study with rabbits. Clinical signs (diarrhea, salivation, lacrimation, and muscular fasciculations) and death were observed in only one oral study at 4.7 and 9.4 mg/kg/day (Doull and Anido, 1957b). Reductions

Table 20. Subchronic Effects of Azinphos-Methyl and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Ref.[a]
		Inhalation			
Rat	6 hrs/day, 5 days/wk, 12 wks	Plasma and RBC[b] ChE[c] inhibition (56-85 %[d])	0.32[e]	1.26	1
		Oral			
Rat[f]	9 days, gavage	Reduced weight gain and food consumption	2.5	5.0	2
Rat[f]	9 days, gavage	Plasma, RBC, and brain ChE inhibition (61-77%)	1.0	2.0	3*
Rabbit[f]	12 days, gavage	Cholinergic signs, brain ChE inhibition (88%)	2.5	6.0	4*
		Plasma ChE inhibition (87%)	1.0	2.5	
		RBC ChE inhibition (86%)	-----	1.0	
Mouse[g]	3-gen., 4-10 wks premating, diet	Mortality and decreased lactation index	3.75	7.5	5
Rat[g]	2-gen., 14 wks premating, diet	Decreased viability and lactation indices	0.33	1.02	6*
Rat[g]	1-gen., 14 wks premating, diet	Plasma and brain ChE inhibition (52-86%), decreased viability index	0.43	1.30	7
		RBC ChE inhibition (53-81%)	-----	0.43	
Rabbit	12 weeks, gavage	Impaired spermatogenesis	-----	1.5	8
Rat	16 weeks, diet	Plasma, RBC, and brain ChE inhibition (60-91%) and decreased weight gain	0.5	1.9	9
Rat	16 weeks, diet	Cholinergic signs, reduced weight gain, plasma, RBC and brain ChE inhibition (25-52%)	-----	4.7	10
Rat	**13 weeks, diet**	**Plasma, RBC and brain ChE inhibition (62-92%)**	**(0.09)[h]**	**0.91**	**11***
Human	30 days, capsule	Plasma and RBC ChE inhibition	0.29	-----	12
		Dermal			
Rabbit	6 hrs/day, 5 days/wk, 3 wks	RBC ChE inhibition (60-77%)	2	20	13

a References: 1. Kimmerle, 1976; 2. Short *et al.*, 1978; 3. Kowalski *et al.*, 1987; 4. Clemens, 1988; 5. Root *et al.*, 1965; 6. Eiben and Janda, 1984; 7. Holzum, 1990; 8. Soliman and El-Zalabani, 1981; 9. Doull and Rehfuss, 1956; 10. Doull and Anido, 1957b; 11. Sheets and Hamilton, 1995; 12. Rider *et al.*, 1972; 13. Flucke and Schilde, 1980.
b RBC = red blood cell
c ChE = cholinesterase
d Percent of control activity
e Estimated assuming a Wistar rat weighs 235 g and breathes 0.05 m^3 in 6 hours (U.S. EPA, 1988).
f Developmental toxicity study: Only maternal effects observed after the first few days were included.
g Reproductive toxicity study
h NOEL estimated by dividing the LOEL by a default uncertainty factor of 10 (Dourson and Stara, 1983).
* Acceptable study based on FIFRA guidelines

in body weights were seen in several studies (Kimmerle, 1976; Doull and Rehfuss, 1956; Doull and Anido, 1957b). The only other effect observed in these studies was a reduction in plasma, RBC and brain ChE activity. The lowest NOEL was 1.24 mg/m^3 (0.32 mg/kg/day) based on the reduction in plasma and RBC ChE activity (56-85 % of control) in the inhalation study (Kimmerle, 1976). However, this study had several deficiencies including no analysis of test article, incomplete clinical chemistry and histopathology.

A subchronic toxicity study in which human volunteers were administered azinphos-methyl in capsules for 30 days was also available (Rider *et al.*, 1972). No effect on clinical signs, hematology, prothrombin time, and urinalysis were observed. No plasma ChE inhibition was observed at doses up to 20 mg/day (~0.29 mg/kg/day). Erratic RBC ChE inhibition was seen at 20 mg/day, but the investigators did not feel this was sufficient to be considered an adverse effect. This study was not considered very useful for risk assessment purposes since only limited information was available with no summary tables or individual data.

In addition to the standard subchronic toxicity studies, Table 20 includes several developmental toxicity studies where maternal effects were observed after repeated, daily exposure to azinphos-methyl for 1 to 2 weeks. Ataxia and tremors were observed in rabbits at 6 mg/kg/day on gestation day 16 (day 10 of exposure). Reduced body weight gains were seen in one rat study (Short *et al.*, 1978). Plasma, RBC and brain ChE activity were reduced in a few studies where it was measured (Kowalski *et al.*, 1987; Clemens *et al.*, 1988). The lowest NOEL for overt toxicity in the developmental toxicity studies was 1 mg/kg/day based on reduced brain ChE activity (61% of controls) in rats (Kowalski *et al*., 1987). The lowest NOEL for blood ChE inhibition was less than 1 mg/kg/day based on reduced RBC ChE activity (86% of controls) in rabbits (Clemens, 1988).

Any effects observed in reproductive toxicity studies were also included in Table 20. The effects observed in the parental generations of the reproductive toxicity studies for azinphos-methyl included death, convulsions, inertia, stumbling gait, nasal discharge, inflammation around eyes, alopecia, impaired spermatogenesis, reduced body weights, reduced ChE activity in plasma, RBC and brain, and hyperemia and edema of the lungs and liver. The effects observed in pups included reduced body weights and survival. The lowest NOEL for overt toxicity in these studies was 5 ppm (F_0M: 0.33 mg/kg/day; F_0F: 0.48 mg/kg/day; F_1BM: 0.42 mg/kg/day; F_1BF: 0.67 mg/kg/day) based on reduced survival of pups (Eiben and Janda, 1984). The lowest NOEL for blood ChE inhibition was less than was 5 ppm (M: 0.43 mg/kg/day; F: 0.55 mg/kg/day) based on reduced RBC ChE activity (53-81% of controls) in adult rats (Holzum, 1990).

One 90-day subchronic neurotoxicity study in rats was available for azinphos-methyl (Sheets and Hamilton, 1995). Tremors, uncoordinated gait, increased reactivity, perianal stain, red lacrimation and oral stain were observed in both sexes at 2.81 mg/kg/day or higher. Reductions in body weight and food consumption were seen in both sexes at 6.99 mg/kg/day or higher. In the FOB, perianal stain, increased reactivity, decreased forelimb grip strength, impaired righting reflex, and tremor were seen primarily in females at 2.81 mg/kg/day or higher. Motor and locomotor activities were significantly reduced in both sexes at 6.99 mg/kg/day or higher. Reduced plasma, RBC and brain ChE activities (62-92% of controls) were the most

sensitive endpoints with a LOEL of 15 ppm (M: 0.91 mg/kg/day;1.05 mg/kg/day in females) in both sexes. This study was selected as the definitive study for evaluating seasonal exposure to azinphos-methyl since it had the most thorough evaluation of neurobehavioral effects and it met FIFRA guidelines. The critical NOEL for subchronic toxicity was estimated to be 0.09 mg/kg/day by dividing the LOEL for reduced plasma, RBC and brain ChE activity by the default uncertainty factor of 10 (Dourson and Stara, 1983).

3. Chronic Toxicity

The effects observed in laboratory animals with chronic exposure to azinphos-methyl are summarized in Table 21. Clinical signs were observed at the higher dose levels in many of the chronic studies including rough hair coat, hyperactivity, convulsions, tremors, exophthalmos (which progressed to unilateral or bilateral blindness), muscular weakness, inactivity, abnormal sitting posterior, diarrhea, mucus in feces, alopecia, and jaundice (1 dog) (NCI, 1978; Schmidt and Chevalier, 1984; Lorke, 1966b; Allen, 1990). Reduced body weights were seen in several studies (NCI, 1978; Schmidt and Chevalier, 1984, Lorke, 1966b). Only a few histopathological lesions were seen including cystic endometrial hyperplasia in one mouse study and cholangitis in one dog (NCI, 1978; Lorke, 1966b). The cholangitis was not considered treatment-related by the investigator because no other hepatic abnormalities, except occasional focus of cellular infiltration, were observed in the other dogs in that study. Plasma, RBC and brain ChE inhibition were the most sensitive endpoints in the chronic studies when they were measured. The lowest established NOEL for overt toxicity in a chronic study was 0.15g/kg/day based on diarrhea in male dogs fed azinphos-methyl in the diet for 1 years (Allen, 1990). The NOEL for RBC ChE inhibition in this study was also 0.15 mg/kg/day. Therefore, the 1-year dog study conducted by Allen (1990) was selected as the definitive study for evaluating chronic exposure to azinphos-methyl with a critical NOEL of 0.15 mg/kg/day for diarrhea and RBC ChE inhibition.

4. Oncogenicity - Weight of Evidence

There was evidence suggesting that azinphos-methyl is oncogenic in two of five oncogenicity studies. There was an increase (19/50 or 38%) in the combined incidence of hepatocellular adenomas and carcinomas in males at the highest dose tested in a mouse study conducted by NCI (NCI, 1978). Interpretation of the findings from the NCI study is difficult because of an inadequate number of concurrent controls (ten mice/sex). The size of the concurrent control group severely reduced the statistical power to detect an increase in tumors. Due to the inadequate number of concurrent controls, the investigators pooled together the control animals from a number of other mouse oncogenicity studies that were currently being conducted at this laboratory for statistical analysis. The increase in liver tumors was statistically significant when compared with pooled controls (30/128 or 23%); however, the investigators did not consider the increase treatment-related since similar high incidences had been observed in other male mice control groups for this same laboratory. No historical control data for these tumors was provided by the investigators, but Ward *et al.* (1979) reported the percent of hepatocellular adenomas and carcinomas to be 7.9 and 13.7%, respectively, in untreated $B6C3F_1$ control mice in NCI studies conducted between 1972 and 1977. Nevertheless, there is no scientific consensus on the use of historical control data in evaluating the toxicological significance of tumor increases in treated animals. No increase in liver tumors or any other

Table 21. Chronic Effects of Azinphos-Methyl and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Ref.[a]
Mouse	80 weeks, diet	Hyperactivity, rough hair coat, cystic endometrial hyperplasia	-----	5.4	1
Mouse	104 weeks, diet	Plasma, RBC[b], and brain ChE[c] inhibition (78-94%[d])	-----	0.79	2*
Rat	97 weeks, diet	Convulsions, RBC and brain ChE inhibition (51-81%)	0.78	3.01	3
		Plasma ChE inhibition (82-90%)	0.21	0.78	
Rat	80 weeks, diet	Reduced body weights	-----	5.7	1
Rat	104 weeks, diet	Plasma, RBC and brain ChE inhibition (65-84%)	0.25	0.75	4*
Dog	2 years, diet	Mortality, cholinergic signs, reduced body weight and food consumption	1.27	4.25	5
		Plasma and RBC ChE inhibition (71-84%)	0.17	1.27	
Dog	**52 weeks, diet**	**Diarrhea, RBC ChE inhibition (65-73%)**	**0.15**	**0.69**	**6***

a References: 1. NCI, 1978; 2. Hayes, 1985; 3. Lorke, 1966a; 4. Schmidt and Chevalier, 1984; 5. Lorke, 1966b; 6. Allen, 1990.
b RBC = red blood cell
c ChE = cholinesterase
d Percent of control activity
* Acceptable study based on FIFRA guidelines

tumors was seen in another mouse oncogenicity study which met FIFRA guidelines (Hayes, 1985). The highest dose level in Hayes study was approximately two-fold lower than the NCI study, but was sufficient to produce a marked reduction in brain ChE activity to approximately 35% of controls. The dose levels in the NCI study may have exceeded criteria for a maximum tolerated dose (MTD) since convulsions were observed at the high dose level during the second year of the study.

In a rat oncogenicity study conducted by NCI, there were increases in tumors of the pituitary, pancreas, thyroid, parathyroid and adrenal glands in males, but the increases were only significant when compared to pooled controls (NCI, 1978). Like the NCI mouse study, the NCI rat study also had an inadequate number of concurrent controls (10 rats/sex) which made interpretation of the findings difficult. Comparison with pooled controls is problematic because the same pathologist did not review the pooled controls and the azinphos-methyl study animals. Furthermore, the toxicological significance of the increase in the pituitary and parathyroid tumors is uncertain since the incidence in the concurrent controls was greater than the pooled controls. The investigators also concluded that the increase in pancreatic and thyroid tumors was not clearly treatment-related because they fell within the historical control range for this laboratory. These data suggest that azinphos-methyl may be oncogenic through some sort of endocrine

disruption; however, a mechanism is not known and no increase in endocrine tumors was seen in two other chronic rat studies, one of which was acceptable based on FIFRA guidelines (Lorke, 1966a; Schmidt and Chevalier, 1984). Several factors may have contributed to the different response in the NCI study compared to the other rat studies including higher dose levels and a different strain of rat. The high dose level in the NCI study was approximately 3-fold higher than the high dose level in the other two rats studies. However, the high dose level in the other two rat studies was high enough to produce significant brain ChE inhibition (45-81% of controls) and, therefore, satisfy the criteria for a MTD. On the other hand, the high dose level in the NCI study may have been exceeded the MTD since cholinergic signs were observed, including tremors and exophthalmos which progressed to unilateral or bilateral blindness. Perhaps the excessive cholinergic stimulation in the NCI study was sufficient to cause endocrine disruption.

Azinphos-methyl appears to be genotoxic based on positive results in several *in vitro* assays including a mouse lymphoma assay, four cytogenetic assays using human cells or cell lines or a hamster cell line, and a micronucleus assay with human lymphocytes (Garret *et al.*, 1986; Herbold, 1989; Alam *et al.*, 1974; Alam and Kasatiya, 1976; Trépanier *et al.*, 1977; Bianchi-Santamaria *et al.*, 1997). However, all the *in vivo* assays were negative including a Drosophila sex-linked recessive lethal assay, a cytogenetic assay in mice, two micronucleus assays in mice, a sister chromatid exchange assay in mudminnows, and four dominant lethal assays in mice. Most of the reverse mutation assays with *Salmonella typhimurium* were also negative except for an equivocal response with the TA100 strain in one study and a weak positive response with the TA98 strain in another study (Lawlor, 1987; Zeiger *et al.*, 1987). The weak positive response was only observed at concentrations where precipitation occurred, confounding the results. All of the other gene mutation assays and miscellaneous genotoxicity tests were negative, except for positive results in a forward mutation assay with *Schizosaccharomyces pombe* ade6 (Gilot-Delhalle *et al.*, 1983), a mitotic recombination assay with *Saccharomyces cerevisiae* D3 (Riccio *et al.*, 1981), a reverse mutation/gene conversion assay with *S.* cerevisiae D7 (Bianchi *et al.*, 1994), a gene conversion/cross-over/non-disjunction assay with *Aspergillus nidulans* D7 (Vallini *et al.*, 1983), and a ^{32}P-postlabeling assay of adducts in calf thymus DNA (Shah *et al.*, 1997).

In analyzing the structural activity relationship of 301 chemicals tested under the U.S. NTP program, Ashby and Tennant (1991) considered chemicals containing an alkyl phosphate ester, such as azinphos-methyl, to be potential alkylating agents. However, they recognized the potential problem alkyl phosphate esters pose in predicting carcinogenicity since 6 of 15 alkyl phosphate esters examined were non-carcinogens and 3 were equivocal carcinogens. Furthermore, 3 alkyl phosphate esters that were considered carcinogens were negative for the *Salmonella* assay. Ashby and Tennant (1991) classified azinphos-methyl as an equivocal carcinogen based on the carcinogenicity study from NCI (1978). They also classified azinphos-methyl as positive for the *Salmonella* assay based on data reported by Zeiger *et al.* (1987) despite the confounding of the results due to the presence of precipitation. They did recommend confirming the mutagenic potential of these alkyl phosphate esters with a chemical alkylating test. The metabolite, benzazimide, did not contain any structural alerts identified by Ashby and Tennant (1991).

The available genotoxicity data for the structurally similar pesticide, azinphos-ethyl, also suggests that it is genotoxic. Azinphos-ethyl was mutagenic in a reverse mutation assay with *Salmonella typhimurium* TA100 strain without metabolic activation, but only weakly mutagenic with activation (Diril *et al.*, 1990). It was not mutagenic with the TA98 strain. Azinphos-ethyl was positive in an *in vitro* micronucleus assay with Chinese hamster lung cells, but negative in an *in vivo* micronucleus assay in mice (Ni *et al.*, 1993). Azinphos-ethyl was also negative for cytogenetic effects in bone marrow cells and spermatogonia from mice exposed *in vivo* and in a dominant lethal assay in mice (Degraeve *et al.*, 1986). Degraeve *et al.* (1986) noted that the high toxicity of azinphos-methyl and azinphos-ethyl may be a limiting factor in demonstrating a cytogenetic effect *in vivo*. Another explanation for the lack of concordance in response between the *in vivo* and *in vitro* cytogenetic assays may be that azinphos-methyl and azinphos-ethyl are quickly metabolized *in vivo* before they can exert any genotoxic effect. No genotoxicity data was available for the metabolite, benzazimide.

In summary, the weight of evidence for oncogenicity is limited for azinphos-methyl. There was an increase in endocrine tumors in several sites in one sex and one strain of rats. There was also an increase in a common tumor (hepatocellular adenomas and carcinomas) in one sex (males) of one strain of mice. However, the findings in both of these studies were compromised by an inadequate number of concurrent controls. The increases in these tumors were only statistically significant when compared with pooled controls. Similar increases in these tumors were not seen in other rat and mouse oncogenicity studies which met FIFRA guidelines. Azinphos-methyl was genotoxic in a number of *in vitro* assays, but not in any *in vivo* assays. Therefore, DPR toxicologists concluded that this limited evidence was insufficient to warrant further evaluation of the oncogenic potential of azinphos-methyl. The U.S. EPA has classified azinphos-methyl as a Group E carcinogen (i.e., no evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiological and animal studies) (Eiden, 1999). In their toxicological evaluation of azinphos-methyl, the Joint Meeting on Pesticide Residues of WHO/FAO concluded that azinphos-methyl had no carcinogenic potential in either rats or mice based on the studies conducted by Hayes (1985) and Schmidt and Chevalier (1984) (JMPR, 1991). In their judgement, these newer studies clarified equivocal evidence in rats in the NCI study. Furthermore, they concluded it was unlikely that azinphos-methyl is genotoxic to humans.

C. Exposure Assessment

1. Offsite Air Exposure

Acute exposure to azinphos-methyl in offsite (application site) air was estimated from air monitoring conducted by the Air Resources Board (ARB) for 5 days following an application to a walnut orchard in Glenn county in July 1994 (For more details see Part B, Exposure Assessment, of the Evaluation of Azinphos-methyl as a Toxic Air Contaminant). Acute exposure was estimated based on the highest residue detected in air samples during one hour of application and approximately 1.5 hours immediately after application (2.2 $\mu g/m^3$ after correction for recovery). Air samples collected after this time were all below the detection limit (0.08 $\mu g/m^3$). The absorbed daily dosages (ADDs) were 170, 80 and 80 ng/kg for children, adult males and adult

Table 22. Estimated Exposure for the General Public to Azinphos-methyl in Offsite and Ambient Air

Population Subgroup	Child	Adult male	Adult female
	Offsite[a]		
ADD[b] (ng/kg)	170	80	80
	Ambient[c]		
ADD (ng/kg)	61.3	23.1	15.7
SADD[d] (ng/kg/day)	11.4	5.1	4.7
AADD[e] (ng/kg/day)	4.7	2.1	1.9

a Offsite exposure dosages based on air concentrations in study by ARB (1995) in Glenn County.
b ADD = Absorbed Daily Dosage using the 95th percentile of the air concentrations. Respiratory uptake and absorption was assumed to be 100%. For more explanation of the calculations, see Part B, Exposure Assessment, in Evaluation of Azinphos-methyl as a Toxic Air Contaminant.
c Ambient exposure dosages based on air concentrations at the Pond site in a study in Kern County by Seiber *et al.* (1988).
d SADD = Seasonal Average Daily Dosage using on the mean air concentration at the Pond site during the monitoring period.
e AADD = Annual Average Daily Dosage assuming the season of potential exposure is 5 months of the year.

females, respectively, assuming a 2.5 hour exposure period and 100% respiratory uptake (Table 22). The air concentration during the rest of the 24-hour period was assumed to be same as the ambient air at the site with the highest air concentration.

2. Ambient Air Exposure

Ambient air monitoring data was collected by Seiber *et al.* (1988) at five rural sites (Pond, two sites in McFarland, Wasco, and Shafter) and one urban site (Bakersfield) in Kern county during June and July of 1987 (For more details see Part B, Exposure Assessment, of the Evaluation of Azinphos-methyl as a Toxic Air Contaminant). The Pond Site represents a worst case exposure scenario because the air sampler was located less than 100 meters from almond orchards to the east, south and west. The distance from orchards at other sites was less than 400 meters. Twenty-four hour air samples were collected 4 days per week for approximately one month. The minimum detection limit ranged from 15 to 43 ng/m^3 depending on the airflow. As expected, the Pond site had the highest average and 95th percentile air concentrations for azinphos-methyl during this monitoring time (26 and 83 ng/m^3, respectively). Therefore, the risk estimates were initially calculated using the exposure estimates from the Pond site, assuming that if they were acceptable at this location, they would be acceptable at the other five locations in Kern County where the air concentrations were lower. The ADDs for the Pond site were 61.3, 23.1, and 15.7 ng/kg/day for a 6-year-old child, an adult male and, an adult female, respectively, using the 95th percentile and 100% respiratory uptake and absorption. The Seasonal Average Daily Dosages (SADDs) were estimated to be 11.4, 5.1. and 4.7 ng/kg/day for children, adult males and adult females, respectively, using the mean ambient air concentration at the Pond site during the one month monitoring period. The Annual Average Daily Dosage (AADD) is the average air concentration for a year assuming the season of potential exposure is 5 months per

year for azinphos-methyl. The AADDs for the Pond site were 4.7, 2.1 and 1.9 ng/kg/day for children, adult males and adult females, respectively.

3. Aggregate Exposure

Dietary and occupational exposure to azinphos-methyl was previously addressed in the Risk Characterization Document (RCD) for this compound. Ambient air exposure to azinphos-methyl in ambient air was not addressed until this document; therefore, aggregate exposure to azinphos-methyl in the diet and ambient air was included in this document. Acute dietary exposure dosages for azinphos-methyl were calculated for the general public in the Exposure Assessment section (section IV.B., pp. 48-55) of RCD. The estimated acute dietary exposure to azinphos-methyl was 6.5, 1.5 and 3.0 µg/kg/day for children (ages 1-6 years old), adult males (13 years and older), and females adults (nursing, 13 years and older – adult female population with the highest dietary exposure), respectively. The estimated chronic dietary exposure was 0.37, 0.12 and 0.16 µg/kg/day for children, adult males and adult females, respectively. The contribution of ambient air exposure to aggregate exposure was negligible since ambient air exposure represented only 1 to 2% of the combined dietary and ambient air exposure to azinphos-methyl. Due to the relatively high exposures in applicators, harvesters and thinners, the contribution of ambient air exposure was even less significant, representing only 0.01 to 0.3% of their aggregate exposure. The contribution to the aggregate exposure was slightly higher (0.2 to 1%) for proppers because their occupational exposure was lower. No further analysis of aggregate exposure was performed due to the small contribution of ambient air to the aggregate exposure for azinphos-methyl.

D. Risk Characterization

The risk for non-oncogenic human health effects is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental animal studies to the human exposure dosage.

$$Margin\ of\ \ Exposure = \frac{NOEL}{Exposure\ Dosage}$$

1. Acute Toxicity

The MOEs for acute exposure to azinphos-methyl were calculated using the acute NOELs from the rat acute neurotoxicity study and the human acute toxicity study and the ADDs for offsite and ambient air in Table 22. Depending on the NOEL selected for evaluating acute exposure, the MOEs for offsite air ranged from 590 for children based on RBC ChE inhibition in rats to 12,000 for adults based on neurobehavioral effects observed in the FOB and plasma and brain ChE inhibition in rats (Table 23). The acute MOEs for ambient air ranged from 1,600 for children based on RBC ChE inhibition in rats to 64,000 in adults based on overt toxicity.

Table 23. Estimated Margins of Exposure for the General Public to Azinphos-methyl in Offsite and Ambient Air[a]

NOEL (mg/kg) (species: endpoints)		**Child**	**Adult Male**	**Adult Female**
		Offsite		
Acute				
1.0	(rat: FOB, plasma/brain ChE)	5,900	12,000	12,000
0.1	(rat: RBC ChE)	590	1,200	1,200
0.75	(human: plasma/RBC ChE)	4,400	9,400	9,400
		Ambient		
Acute				
1.0	(rat: FOB, plasma/brain ChE)	16,000	43,000	64,000
0.1	(rat: RBC ChE)	1,600	4,300	6,400
0.75	(human: plasma/RBC ChE)	12,000	32,000	48,000
Seasonal				
0.09	(rat: plasma/RBC/brain ChE)	7,900	18,000	19,000
Chronic				
0.15	(dog: diarrhea, RBC ChE)	32,000	71,000	79,000

a Margin of Exposure = NOEL / Exposure Dosage. Exposure dosages are from Table 22. Values rounded to two significant figures.

2. Subchronic Toxicity

The MOEs for seasonal exposure to azinphos-methyl were calculated using the estimated subchronic NOEL of 0.09 mg/kg/day and the SADDs for ambient air at the Pond site. The subchronic MOEs ranged from 7,900 for children to 19,000 for adult females.

3. Chronic Toxicity

The MOEs for chronic exposure to azinphos-methyl were calculated using the chronic NOEL of 0.15 mg/kg/day and the AADDs for ambient air at the Pond site. The MOEs for chronic exposure to azinphos-methyl in ambient air ranged from 32,000 for children to 79,000 for adult females.

IV. RISK APPRAISAL

A. Introduction

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects observed in toxicity studies with laboratory animals will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. This, in turn, results in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for azinphos-methyl are delineated in the following discussion.

B. Hazard Identification

The most sensitive endpoint with acute, subchronic and chronic exposure to azinphos-methyl was ChE inhibition. Although the physiological role of AChE in the nervous system is well known, there is some uncertainty regarding the toxicological significance of brain ChE inhibition because of the poor correlation between the severity of cholinergic signs and the level of ChE inhibition in the brain (U.S. EPA, 1988b). Several factors probably contribute to the poor correlation. One of these factors is that ChE inhibitors produce different degrees of inhibition in the various regions of the brain (Nieminen *et al.*, 1990). Certain cholinergic signs may be due to inhibition in specific regions of the brain. The level of brain ChE inhibition required to produce these effects may not be representative if the activity is measured in the whole brain or regions of the brain that are insensitive to ChE inhibitors. Another factor is that some cholinergic signs may be due to peripheral rather than central inhibition of AChE (Murphy, 1986). For example, some of the respiratory effects may be due to peripheral inhibition of AChE in the diaphragm resulting in paralysis. In addition, brain ChE activity is usually measured at the end of the study whereas the cholinergic signs may be observed at various time points during the study. Often cholinergic signs are observed only at the beginning of the study and then the animals appear to develop a "tolerance" to the ChE inhibitor. This adaptation or "tolerance" may be due to several possible mechanisms including down-regulation of post-synaptic receptors (Costa *et al.*, 1982). Finally, clinical observation in animal studies is a very crude and subjective measurement. Some mild cholinergic symptoms, such as headaches and anxiety, cannot readily be detected in animals. The clinical signs in animals can also be missed because of the timing of the observations, especially with reversible ChE inhibitors. Rodents are nocturnal and generally eat and drink at night. If a chemical is a reversible inhibitor, some of the cholinergic signs could be missed because the signs occurred shortly after the animals had eaten during the night. There may also be other subtle changes in neurological function that will only be detected if the animal is stressed or required to perform certain tasks (Nagymajtényi *et al.*, 1988; Raffaele and Rees, 1990). It is possible that some level of brain ChE inhibition can occur without any untoward effect on neurological function, overt or subtle. However, the only way to be certain of this is

through rigorous behavioral and neurophysiological testing in animals or humans. Although some neurobehavioral testing was conducted (FOB and motor activity) with acute exposure to azinphos-methyl, no tests for memory or learning deficits were performed. Nor were there any tests for subtle neurological effects with subchronic or chronic exposure to azinphos-methyl. Therefore, the assumption was made that since there was a statistically significant inhibition of brain ChE inhibition, there was probably some deleterious effect to the neurological system.

A NOEL for overt toxicity of 1 mg/kg from an acute rat neurotoxicity study was identified for evaluating acute exposure to azinphos-methyl in humans based on effects observed in a FOB (sitting or lying in open field, reduced approach response and uncoordinated righting response) and brain ChE inhibition (49% of controls) in females (Sheets, 1994). Both of these endpoints are of uncertain toxicological significance. As mentioned above, the brain ChE inhibition was assumed to be toxicologically significant because of the lack of testing for learning and memory deficits. The performance in the FOB is also uncertain because the differences were not statistically significant, but they were assumed to be toxicologically significant because only 3 of 18 female survived at 6 mg/kg. Therefore, it is possible the NOEL is higher than assumed. However, the LOEL of 3 mg/kg in this study was similar to the LOELs observed in two rat LD_{50} studies, 2.0 and 2.5 mg/kg (Crawford and Anderson, 1974; Mihail, 1978). These studies were not used because the reports were so brief that the clinical signs were not described for each dose level and the studies did not meet FIFRA guidelines. Higher NOELs of 2.5 mg/kg were reported in several other acute oral toxicity studies; however, only one of these studies, a rabbit developmental toxicity study, did not have major deficiencies (Hecht, 1955; Short *et al.*, 1978; Clemens *et al.*, 1988). The NOEL in the rabbit study was based on an increase in pre- and post-implantation losses. The toxicological significance of this endpoint is also uncertain because they were not observed in two other range-finding studies where rabbits were administered azinphos-methyl at equal or higher dose levels. However, the number of animals per dose was too small (2-4 animals/dose) in both studies to allow meaningful statistical analysis.

A NOEL of 0.1 mg/kg/day was estimated for blood ChE inhibition from the acute neurotoxicity study in rats based on a slight reduction of RBC ChE activity in females (83% of controls) at 1 mg/kg/day (Sheets, 1994). This NOEL was estimated by dividing the LOEL by the standard uncertainty factor of 10. However, the LOEL may be very close to the NOEL given that the reduction in RBC ChE activity was less 20% from controls and the dose response curve for azinphos-methyl appears to be very steep since the majority of females (15/18) at 6 mg/kg died. A more realistic estimate of the NOEL for RBC ChE inhibition may be obtained by dividing the LOEL by an uncertainty factor of 3 rather than 10. A higher NOEL for RBC ChE inhibition is supported by higher observed NOELs for plasma and RBC ChE inhibition in numerous subchronic and chronic studies including a 3-month inhalation study in rats, a developmental toxicity study in rats, a 16-week feeding study in rats, and a 2-year feeding study in rats (Kimmerle, 1976; Kowalski *et al.*, 1987; Doull and Rehfuss, 1956; Schmidt and Chevaleir, 1984).

A NOEL for plasma and RBC ChE inhibition of 0.75 mg/kg was established in a study with male and female human volunteers (McFarlane and Freestone, 1998). No effects, including

ChE inhibition, were observed at the highest dose level tested in males and females (1.0 and 0.75 mg/kg, respectively). Because no effects were observed at the highest dose levels tested, the NOEL could be higher. On the other hand, the subjects were not evaluated for neurophysiological or cognitive function, so its possible some subtle effect could have been overlooked. However, neurological effects were only observed in animals at dose levels that resulted in significant brain ChE, so it seems unlikely that effects would be seen at dose levels below that which caused significant plasma and RBC ChE inhibition. The NOEL from this study is higher than the absorbed NOEL of 0.3 mg/kg/day that Carrier and Brunet (1999) estimated from urinary metabolite data in peach harvesters. The NOEL from the McFarlane and Freestone study was considered more reliable because the exposure was controlled and subjects were more thoroughly evaluated for neurological effects. However, if the NOEL from the Carrier and Brunet (1999) study had been used to evaluate acute exposure, the MOEs would be approximately 2.5 times lower than estimated.

The subchronic neurotoxicity study in rats was selected as the definitive study for evaluating seasonal exposure to azinphos-methyl (Sheets and Hamilton, 1995). A NOEL was not established for plasma, RBC or brain ChE inhibition in this study. Therefore, it was estimated by dividing the LOEL, 0.91 mg/kg/day, by the default uncertainty factor of 10. However, the actual NOEL is probably closer to the observed NOEL of 0.25 mg/kg/day for the same endpoints in the 2-year rat study (Schmidt and Chevalier, 1984). If the NOEL for the chronic rat study was used for evaluating seasonal exposure to azinphos-methyl in ambient air, the MOEs would be approximately 30% greater than estimated.

Carrier and Brunet (1999) estimated an absorbed NOEL for repeated exposure of 0.1 mg/kg/day. This estimated NOEL was not used for evaluating seasonal exposure to azinphos-methyl despite being based on human data because the exposure was not controlled. Instead exposure was estimated based on urinary metabolite data using a toxicokinetic model. This approach would add additional uncertainty to the risk calculations. Furthermore, this NOEL is similar to the NOEL of 0.09 mg/kg/day which was used in the calculation of the seasonal MOEs for azinphos-methyl in ambient air, so that the outcome would not have been much different.

While brain ChE inhibition was one of the more sensitive endpoints for overt toxicity for azinphos-methyl in most studies, it does appear to be the most sensitive endpoint in one chronic dog study that was used for evaluating chronic exposure (Allen, 1990). An increase in diarrhea and mucus in the feces was observed in males at a dose level which did not produce significant brain ChE inhibition. Although the increase in males did not exhibit a clear dose-response, a health protective assumption was made that the increase in frequency in males at 25 ppm was treatment-related and the NOEL was set at 5 ppm (M: 0.15 mg/kg; F: 0.16 mg/kg). If only the diarrhea in the females at 125 ppm was considered treatment-related, then the NOEL for overt toxicity would be 25 ppm (M: 0.69 mg/kg/day; F: 0.78 mg/kg/day) based on the diarrhea, and plasma and brain ChE inhibition. The NOEL for RBC ChE inhibition would still be 5 ppm (M: 0.15 mg/kg/day; F: 0.16 mg/kg/day). If the higher NOEL for overt toxicity was used for this study, then the NOEL from the rat chronic toxicity study (M: 0.25 mg/kg/day; F: 0.31 mg/kg/day) would have the lowest NOEL for overt toxicity (Schmidt and Chevalier, 1984). If the NOEL from the rat chronic toxicity study had been used to evaluate chronic exposure to azinphos-

methyl in ambient air, then the chronic MOEs would be approximately 65% higher than estimated.

It would be preferable to use a NOEL from an inhalation study to evaluate the potential health effects from exposure to azinphos-methyl in ambient air. Three inhalation studies were available for azinphos-methyl which were not used because of deficiencies with the studies. In a 4-hour inhalation LC_{50} study (whole body), a NOEL of 23 mg/m^3 (4.1 mg/kg) was reported based on unspecified signs of toxicity at 59 mg/m^3 in male rats (Kimmerle, 1966). In another 4-hr inhalation LC_{50} study (head only), all of the female rats at the lowest dose tested, 80 mg/m^3 (14.4 mg/kg) exhibited cholinergic signs (ocular and nasal discharge, salivation, hypoactivity, tremors, and/or twitching) (Shiotsuka, 1987). A NOEL of 1.4 mg/kg could be estimated for this study by dividing the lowest-observed-effect level (LOEL) by an uncertainty factor of 10. If the NOELs for overt toxicity from these other studies had been used, the acute MOEs would be approximately 1.4 to 4.1 times larger than estimated using the NOEL for overt toxicity from the oral rat neurotoxicity study (Sheets, 1994). The NOEL of 1.26 mg/kg/day for overt toxicity from the 3-month inhalation study could have also been selected as the critical NOEL for evaluating seasonal exposure (Kimmerle, 1976). The NOEL for plasma and RBC ChE inhibition in this study was even lower at 0.32 mg/kg/day. However, this study did not evaluate the neurobehavioral effects of azinphos-methyl as thoroughly as the subchronic neurotoxicity study. In addition, this study had no analysis of the test article, incomplete clinical chemistry and histopathological examination and no individual data. If the NOEL for overt toxicity from the subchronic inhalation study had been used instead of the estimated NOEL from the subchronic neurotoxicity study (Sheets and Hamilton, 1995), the subchronic MOEs would be more than 12 times larger than estimated. If the NOEL for plasma and RBC ChE inhibition from the subchronic inhalation study had been used instead of the estimated NOEL from the subchronic neurotoxicity study, the subchronic MOEs would be approximately 3 times larger than estimated.

C. Exposure Assessment

Uncertainties associated with the exposure assessment are discussed in detail in Part B, Exposure Assessment, of the Evaluation of Azinphos-methyl as a Toxic Air Contaminant. The uncertainties include inhalation absorption of azinphos-methyl, indoor air concentrations of azinphos-methyl, dermal exposure from airborne azinphos-methyl, air concentrations throughout season of use, and air concentrations of azinphos-methyl since 1987.

D. Risk Characterization

Generally, an MOE of at least 100 is considered sufficiently protective of human health when data is derived from animal studies. The MOE of 100 allows for humans being 10 times more sensitive than animals and for the most sensitive human being 10 times more sensitive than the least sensitive human. When the NOEL is derived from a human study, then a MOE of at least 10 is generally considered sufficiently protective. The MOEs for acute exposure to azinphos-methyl in offsite air were greater than 500 for both children and adults when based on RBC ChE inhibition in rats. However, when the NOEL for plasma and RBC ChE inhibition in humans was used, the MOEs for offsite air were greater than 4,000 for children and adults. This

suggests that rats may be more sensitive than humans with respect plasma and RBC ChE inhibition. Therefore, a 10-fold uncertainty factor for interspecies differences may not be necessary when determining an acceptable exposure level for azinphos-methyl in humans. The MOEs for acute exposure to azinphos-methyl in ambient air were greater than 1,000 for population subgroups regardless of the NOEL used. The MOEs for seasonal exposure to azinphos-methyl in ambient air were greater than 7,000 for all population subgroups based on plasma, RBC and brain ChE inhibition in rats. The MOEs for chronic exposure to azinphos-methyl in ambient air were greater than 30,000 for both adults and children based on plasma, RBC and brain ChE inhibition in rats.

E. U.S. EPA=s Human Health Risk Assessment for Azinphos-methyl

U.S. EPA completed a Human Health Risk Assessment document for azinphos-methyl in May 1999 (Eiden, 1999). U.S. EPA did not estimate exposure to the general public from azinphos-methyl in the ambient air. However, they did evaluate inhalation exposure in workers using the 3-month inhalation study conducted by Kimmerle (1976) which was not used by DPR toxicologists because of deficiencies including no analysis of test article, incomplete clinical chemistry and histopathological examination and no individual data. The only findings in this study were a slight body weight reduction in males (~8%) and plasma and RBC ChE in both sexes at the highest dose level, 4.72 mg/m^3 (1.26 mg/kg/day). DPR toxicologists considered these findings to be of uncertain toxicological significance and set the NOEL for overt toxicity at 4.72 mg/m^3. The NOEL for plasma and RBC ChE inhibition was 1.24 mg/m^3 (0.32 mg/kg/day). U.S. EPA used the NOEL for plasma and RBC ChE inhibition as a surrogate for peripheral ChE inhibition to evaluate both short-term and intermediate inhalation exposure in workers. If DPR had used this NOEL for evaluating exposure to azinphos-methyl in ambient air, the acute MOEs would range from 2,700 (child, offsite) to 20,000 (adult female, ambient). The seasonal MOEs would range from 28,000 (child) to 68,000 (adult female).

U.S. EPA agreed with DPR=s analysis of the developmental and reproductive toxicity studies in that there was no evidence of increased pre- or postnatal sensitivity to azinphos-methyl and did not recommend an additional uncertainty factor of 10X be used under FQPA. In addition, U.S. EPA agreed with DPR=s analysis of the weight of evidence for oncogenicity and classified azinphos-methyl as a Group E carcinogen or Anot likely@ to be a human carcinogen.

F. Issues Related to the Food Quality Protection Act

The Food Quality Protection Act of 1996 mandated U.S. EPA to “upgrade its risk assessment process as part of the tolerance setting procedures” (U.S. EPA, 1997a and b). The improvements to risk assessment were based on the recommendations from the 1993 National Academy of Sciences report, “Pesticides in the Diets of Infants and Children” (NAS, 1993). The Act required an explicit finding that tolerances are safe for children. U.S. EPA was required to use an extra 10-fold safety factor to take into account potential pre- and post-natal developmental toxicity and the completeness of the data unless U.S. EPA determined, based on reliable data, that a different margin would be safe. In addition, U.S. EPA must consider available information on: 1) aggregate exposure from all non-occupational sources; 2) effects of cumulative exposure

to the pesticide and other substances with common mechanisms of toxicity; 3) the effects of *in utero* exposure; and 4) the potential for endocrine disrupting effects.

1. Pre- and Post-natal Sensitivity

Developmental toxicity studies in rats and rabbits and reproductive toxicity studies in rats were considered in assessing the potential for greater sensitivity in infants and children than adults. Two developmental toxicity studies were conducted for azinphos-methyl which met FIFRA guidelines, one in rats and the other in rabbits (Kowalski *et al.*, 1987; Clemens *et al.*, 1988. No treatment-related increases in fetal malformations or variations were observed in rats and rabbits in these studies. Maternal effects were primarily brain ChE inhibition. In rats, the maternal brain ChE activity was reduced (73% of controls) at 2.0 mg/kg/day on day 20 of gestation; however, fetal brain ChE activity was unaffected. In rabbits, brain ChE activity was reduced to 88% of controls in does at 6 mg/kg/day on day 28. Ataxia and tremors were also observed in the does at 6 mg/kg/day. A slight increase in pre- and post-implantation losses was seen at 6 mg/kg/day; however, brain ChE activity was not measured in fetuses. These findings in rats and rabbits suggest there is no increased prenatal sensitivity to azinphos-methyl.

An acceptable reproductive toxicity study was available in which azinphos-methyl was administered in the feed to rats (Eiben and Janda, 1984). Several signs were observed in adults at 45 ppm, including alopecia, inflammation of the eyes, convulsions, and death. Four of the 5 deaths occurred in females during lactation. The convulsions were also seen primarily in females. The investigators attributed the increased convulsions and death in females to increased consumption of feed during gestation and lactation. Brain ChE activity was not measured in this study; however, in a 28-day range-finding study conducted in the same laboratory, brain ChE activity was reduced at 20 ppm (82% of controls) at the study termination (Eiben *et al.*, 1983). Based on a range-finding study, DPR toxicologists concluded the NOEL for the reproductive toxicity study was 5 ppm (0.4 mg/kg/day). There was a slight reduction in pup survival to day 4 and day 21 (11% and 8%, respectively) at 15 ppm in one generation, but not both. Based on the reduced pup survival, DPR toxicologists concluded the reproductive NOEL was also 5 ppm. Although brain ChE activity was not measured in pups, these data suggests there is no increased postnatal sensitivity to azinphos-methyl.

2. Endocrine Effects

The Food Quality Protection Act (FQPA) of 1996 required U.S. EPA to develop a screening program to determine the endocrine disruption potential of pesticides. In 1997, the Risk Assessment Forum of the U.S. EPA published a report that reviewed the current state of science relative to environmental endocrine disruption (U.S. EPA, 1997c). U.S. EPA formed the Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) to develop a strategy for screening and testing of pesticides for their potential to produce endocrine disruption. The EDSTAC members include various stakeholders and scientific experts. This screening and testing process is expected to be implemented by August of 1999 as required by FQPA.

Environmental chemicals can interact with the endocrine system, resulting in cancer, reproductive and/or developmental anomalies (EDSTAC, 1998). It may produce these effects by affecting hormonal production and synthesis, binding directly to hormone receptors or interfering with the breakdown of hormones (U.S. EPA, 1997c). The interim science policy stated in U.S. EPA's 1997 report is that "*the Agency does not consider endocrine disruption to be an adverse endpoint per se, but rather to be a mode or mechanism of action leading to other outcomes.*" Possible endocrine-related effects were seen in several reproductive toxicity studies for azinphos-methyl, including reductions in viability and lactation indices and impaired spermatogenesis (Root *et al.*, 1965; Eiben and Janda, 1984; Holzum, 1990; Soliman d El Zalabani, 1981). Other possible endocrine-related effects were seen in one oncogenicity study in rats where an increase in tumors of the pituitary, pancreas, thyroid, parathyroid and adrenal glands were seen in males (NCI, 1978). However, it is unclear from these data if these effects are mediated through endocrine disruption, ChE inhibition or some other mechanism.

3. Cumulative Toxicity

The toxicity of azinphos-methyl may be underestimated if people are exposed simultaneously with other organophosphates, such as, DDVP, diazinon, disulfoton, etc., which have been shown to have a synergistic effect on the acute toxicity of azinphos-methyl in laboratory animals (DuBois, 1962b; DuBois, 1958; McCollister *et al.*, 1968; Witherup and Schlecht, 1963). Synergism between organophosphates is not uncommon, although the exact mechanism of this synergism is uncertain (Murphy, 1986). One possible mechanism is the inhibition the carboxylesterase enzymes that are involved in the detoxification of some organophosphates. Another mechanism could be competition for non-vital binding sites which may act as a buffer, thereby protecting AChE. U.S. EPA is currently in the process of developing the methodology to address this issue.

4. Aggregate Exposure

Combined dietary and ambient air exposure in the general population have been addressed in this document under the Exposure Assessment section in Risk Analysis section. In addition, combined dietary, occupational and ambient air exposure in workers has also been addressed. The contribution of the ambient air exposure to dietary and especially occupational exposure was so small that no further analysis of aggregate exposure was performed.

V. REFERENCE CONCENTRATIONS

Air concentrations of azinphos-methyl below the reference concentrations (RfCs) are generally considered sufficiently low to protect human health. RfCs were calculated for azinphos-methyl for acute, seasonal and chronic exposures. The NOELs from oral studies were converted to equivalent human inhalation NOELs by dividing the oral NOELs by the respiratory rate for humans.

$$human\ inhalation\ NOEL\ (mg/m^3) = \frac{oral\ NOEL\ (mg/kg)}{respiratory\ rate_{human}\ \ (kg/m^3)}$$

Since children have the highest respiratory rate for humans relative to their body weight, their respiratory rate was used for humans. The resulting equivalent acute human inhalation NOELs were 1.35, 0.135, and 1.01 mg/m^3 based on rat overt toxicity, rat RBC ChE inhibition, and human plasma and RBC ChE inhibition, respectively, assuming a 24-hr respiratory rate of 0.74 m^3/kg for a 6-year old child. The equivalent human inhalations NOELs for subchronic and chronic toxicity were 0.135 and 0.378 mg/m^3, respectively, based on rat plasma, RBC and brain ChE inhibition. The RfCs were then calculated by dividing the equivalent human inhalation NOELs for acute and chronic toxicity by an uncertainty factor of 100 when based on a NOEL from an animal study to account for interspecies and intraspecies variation in susceptibility. When the NOEL is from a human study the RfC is calculated by dividing by an uncertainty factor of only 10 for intraspecies variation in sensitivity.

$$RfC\ (mg/m^3) = \frac{human\ inhalation\ NOEL\ (mg/m^3)}{uncertainty\ \ factor\ (e.g.,100)}$$

$$RfC\ (ppm) = RfC\ (mg/m^3)\ x\ \frac{M.Vol.(24.5\ L\ @\ 25^oC)}{M.Wt\ (317.3\ g).}$$

The resultant RfCs for acute exposure (24-hour) are 13.5 µg/m^3 (1.04 ppb), 1.3 µg/m^3 (0.10 ppb), and 101 µg/m^3 (7.8 ppb) based on rat overt toxicity, rat RBC ChE inhibition and human plasma and RBC ChE inhibition, respectively. The highest 24-hour concentration detected in the monitoring of azinphos-methyl in ambient air was 0.11 µg/m^3 (8.4 ppt) at the Pond site. The highest air concentration detected in offsite monitoring was 2.2 µg/m^3 (0.17 ppb) during a 3-hour monitoring interval during and 1 hour after application. Using the detection limit of 0.08 µg/m^3 for the remainder of the day, the 24-hour average air concentration was equivalent to 0.34 µg/m^3 (26 ppt). The RfC for seasonal exposure to azinphos-methyl is 1.2 µg/m^3 (0.09 ppb) based on plasma, RBC and brain ChE inhibition in rats. The average air concentration at the Pond site during the one-month monitoring period was 26 ng/m^3 (2.0 ppt). The RfC for chronic exposure is 2.0 µg/m^3 (0.16 ppb) based on diarrhea and RBC ChE inhibition in dogs. Assuming the season for azinphos-methyl use lasts 5 months, the annual average air concentration at the Pond site would be 1.0 ng/m^3 (0.8 ppt).

VI. CONCLUSIONS

Several acute NOELs were selected for evaluating exposure to azinphos-methyl in offsite and ambient air. The selected acute NOELs were 1.0, 0.1, and 0.75 mg/kg for overt toxicity in rats, RBC ChE inhibition in rats, and plasma and RBC ChE inhibition in humans, respectively. The subchronic NOEL selected for evaluating exposure to azinphos-methyl in ambient air were 0.09 mg/kg/day based on plasma, RBC and brain ChE inhibition in rats. The chronic NOEL selected was 0.15 mg/kg/day based on diarrhea and RBC ChE inhibition in dogs. Six-year-old children have the highest breathing rate per body weight; therefore, they had the highest estimated acute, seasonal and chronic exposure. The estimated acute exposures to azinphos-methyl in offsite and ambient air were 170 and 61.3 ng/kg/day, respectively, for a 6-year-old child. The estimated seasonal and chronic exposure to azinphos-methyl in ambient air was 11.4 and 4.7 ng/kg/day, respectively, for a 6-year-old child. The MOEs for acute exposure to azinphos-methyl in offsite and ambient air ranged from 590 to 64,000 depending on the NOEL used and the population subgroup. The MOEs for seasonal and chronic exposure were 7,900 and 32,000, respectively, for 6-year-old children. The acute RfCs for azinphos-methyl range from 1.3 $\mu g/m^3$ (0.10 ppb) to 101 $\mu g/m^3$ (7.8 ppb) depending on which NOEL was used. The seasonal RfC is 1.2 $\mu g/m^3$ (0.09 ppb) based on plasma, RBC and brain ChE inhibition in rats. The chronic RfC is 2.0 $\mu g/m^3$ (0.16 ppb) based on diarrhea and RBC ChE inhibition in dogs.

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